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Tra. 11 PATENTEIYII Eve: 379 FASENACIONALII
Act. 400 OPOSIOBSERVTER Folios: 87

FORMULARIO ÚNICO DE OPOSICIÓN

①

OPOSICIÓN A:

- | | |
|---|---|
| ☒ Patente de Invencción | ☐ Marca Colectiva |
| ☐ Patente de Modelo de Utilidad | ☐ Marca de Certificación |
| ☐ Diseño Industrial | ☐ Lema Comercial |
| ☐ Esquema de Trazado para Circuitos Integrados | |
| ☐ Marcas de Productos o Servicios | |

②

Número del expediente: 09-030.399

Solicitante: CENTRO DE INMUNOLOGIA MOLECULAR

SOLICITUD

PUBLICADA

Apoderado: CASTELLANOS ABONDANO MARGARITA

Título de la nueva creación o denominación del signo: COMPOSICIONES

TERAPEUTICAS PARA POTENCIAR EL EFECTO DE LA TERAPIA CON ANTICUERPOS CONTRA EL RECEPTOR DEL FACTOR DE CRECIMIENTO EPIDERMICO

Gaceta: 636

③

OPOSICIÓN PRESENTADA POR:

SOLICITANTE	Nombre: PROCAPS S.A.	IDENTIFICACIÓN	
	Dirección: CALLE 80 No. 78b-201	C.C. ☐	NIT ☒
	Nacionalidad o Domicilio: COLOMBIA	C.E. ☐	Otro ☐
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REPRESENTANTE O APODERADO	Nombre: ELIANA ISAURA MORENO	IDENTIFICACIÓN	
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		Número	51'975.664 TP 83823

④

FUNDAMENTOS DE LA OPOSICIÓN:

Art. 14, 15, 18, 20 y 30 de la Decisión 486 de la CAN

Causal: No cumple con los requisitos de patentabilidad.

Signo opositor:

Justificación:

La presente solicitud no cumple con los requisitos de patentabilidad dado que lo revelado, ya se encontraba en el estado de la técnica o es posible derivarlo de la misma tal y como se demostrará en el capítulo "Fundamento de Hechos".

⑤ Comprobante de pago No. _____

Fecha _____

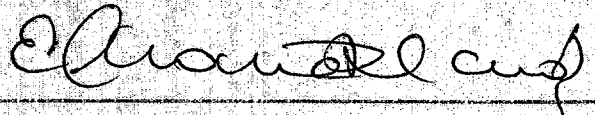
⑥ ANEXOS

- ☒ Comprobante de pago de la tasa.
- ☐ Poderes, si fuere el caso.
- ☒ Pruebas de los fundamentos de la oposición.
- ☐ Documentos que acrediten el legítimo interés, si fuere el caso.
- ☐ Documento que acredita la existencia y representación legal de las partes, cuando sean personas jurídicas.

⑦

NOMBRE: ELIANA ISAURA MORENO BOHORQUEZ

FIRMA: _____



C.C. 51'975.664

TP. 83823

Señora Jefe,

DIVISIÓN DE NUEVAS CREACIONES

Superintendencia de Industria y Comercio

E. S. D.

Referencia : Expediente N° 09-30399

Asunto : Oposición a la solicitud de patente de Invención titulada
*"COMPOSICIONES TERAPEUTICAS PARA POTENCIAR
EL EFECTO DE LA TERAPIA CON ANTICUERPOS
CONTRA EL RECEPTOR DEL FACTOR DE
CRECIMIENTO EPIDERMICO"*

Solicitante : CENTRO DE INMUNOLOGIA MOLECULAR.

Opositora : PROCAPS S.A.

Publicada en la Gaceta de la Propiedad Industrial

N° 616 del 20 de mayo de 2010

Yo, *ELLANA ISAURA MORENO BOHORQUEZ*, abogada en ejercicio, identificada como aparece al pie de su firma, con tarjeta profesional No. 83823 del Consejo Superior de la Judicatura, en mi condición de agente oficioso de la sociedad *PROCAPS S.A.* conformada bajo las leyes de la República de Colombia y domiciliada en la ciudad de Barranquilla, Colombia, atentamente manifiesto que estando dentro del término de ley y por orden expresa de mi agenciado, presento oposición a la concesión del registro de la Patente de Invención titulada *"COMPOSICIONES TERAPEUTICAS PARA POTENCIAR EL EFECTO DE LA TERAPIA CON ANTICUERPOS CONTRA EL RECEPTOR DEL FACTOR DE CRECIMIENTO EPIDERMICO"*, cuyo titular es

CENTRO DE INMUNOLOGIA MOLECULAR, y pido a Usted declarar fundada esta oposición y en consecuencia negar el registro de la patente solicitada.

1. INTERÉS PARA ACTUAR

Conforme a lo dispuesto en el artículo 42 de la Decisión 486 de 2000 de la CAN, estando dentro del término estipulado por la ley, manifiesto el legítimo interés de nuestra representada para oponerse al registro de la patente de la referencia, atendiendo a que la solicitud pretendida no cumple con los requisitos dispuestos en la ley como se analizará más adelante y de ser concedida afectaría directamente la comercialización de algunos productos de mi representada, que contienen, en particular **anticuerpos**. Por lo tanto, solicito respetuosamente a ese Despacho se sirva tener en cuenta la presente oposición dentro del trámite administrativo que se surte en esta Superintendencia.

2. FUNDAMENTOS DE HECHOS

2.1. La solicitud objeto de la presente fue presentada el 25 de marzo de 2009, reivindicando prioridad bajo la solicitud CU20060000190 del 2006-09-29; y fue publicada en Colombia, en la Gaceta de la Propiedad Industrial N° **616 del 20 de mayo de 2010**, cuya publicación internacional corresponde a la WO2008037225 del 2008-04-03.

2.2. La solicitud colombiana **09-30399** objeto de la presente oposición, se refiere a una composición terapéutica útil en el tratamiento del cáncer caracterizada porque comprende la administración simultánea o secuencial de un anticuerpo contra el

receptor del EGF y uno o más interferones del tipo 1; donde la composición terapéutica está caracterizada porque comprende un anticuerpo contra el receptor del EGF, interferón y un excipiente adecuado para su administración por vía intravenosa; además por que el anticuerpo monoclonal contra el receptor del EGF es un anticuerpo quimérico; donde el anticuerpo monoclonal contra el receptor del EGF es un anticuerpo humanizado; donde el anticuerpo monoclonal contra el receptor del EGF es el anticuerpo humanizado hR3 producido por la línea celular con número de depósito 951110101; donde el interferón es de tipo 1; donde el interferón es INF-a humano; donde el interferón es INF- a humano recombinante; y donde el anticuerpo monoclonal HR3 contra el receptor de EGF producido por la línea celular con no. de Depósito 951110101 y el interferón INF-a humano recombinante.

2.3.En primera instancia, se debe advertir que las reivindicaciones se refieren al uso y a un método de tratamiento y no sólo a la composición farmacológica o a un método de fabricación mediante la cual se obtenga un producto tangible y según la legislación, decisión 486 de la Comunidad Andina de Naciones, Artículo 14.- *“Los Países Miembros otorgarán patentes para las invenciones, sean de producto o de procedimiento (...)”*, los usos no están contemplados como patentables; y, Artículo 20.- *“No serán patentables (...) d) los métodos terapéuticos o quirúrgicos para el tratamiento humano o animal, así como los métodos de diagnóstico aplicados a los seres humanos o a animales”*. Solo serán patentables los productos o procedimientos. Por lo tanto a luz de la norma, dicha materia referente al uso y a un método de tratamiento no sería patentable.

2.4.Ahora bien, con base en el artículo 14 de la Decisión 486 de la CAN consagra lo siguiente: *“Los países miembros otorgarán patentes para las invenciones, sean de producto o procedimiento, en todos los campos de la tecnología, siempre que sean nuevas, tengan nivel inventivo y sean susceptibles de aplicación industrial”*. Así las cosas, las invenciones

objeto de patente deben ser nuevas y tener nivel inventivo. Bien, pues antes de la fecha de prioridad de esta solicitud se encuentran los siguientes documentos que afecta la patentabilidad de la presente solicitud:

- D1: WO02089842 publicada el 14 de noviembre de 2002.
- D2: US2006134064 publicada el 22 de junio de 2006.

2.5. Ahora bien, el documento D1, particularmente en la descripción revela una composición terapéutica para el tratamiento del cáncer que comprende un anticuerpo monoclonal (AbM) específico contra el Receptor del Factor de Crecimiento Epidérmico (EGFR) y un agente anti-hormonal. En D1 se describe una composición terapéutica para el tratamiento del cáncer que comprende un anticuerpo monoclonal anti-EGFR, agentes anti-hormonales y, opcionalmente, un antagonista de la angiogenesis, como el INF- α (cf.: D1: pagina 10, líneas 9-15; pagina 16, línea 5; reivindicaciones 1 y 5). Ahora bien, el documento D2 revela una composición terapéutica para el tratamiento del cáncer que comprende el Interferón-alfa (INF- α) y un inhibidor de la actividad tirosinquinasa del EGFR; y, en particular, ha descrito la composición terapéutica que comprende un inhibidor específico de la actividad TK del EGFR, el INF- α , antagonista de la angiogenesis, y, opcionalmente, un AbM anti-HER2 ('Trastuzumab') que se une específicamente al receptor HER2 perteneciente a la familia de TKs del EGFR (cf.: D2: pagina 4, párrafos 0035-0037). Por lo tanto, visto dicho documento D2 en combinación con el documento D1, la presente solicitud, sobre la base de la información técnica divulgada en D1 y D2, combinada con los conocimientos de uso y aplicación habitual en este campo de la biotecnología, se concluye que habría que esperar razonablemente que el experto en la materia estaría confiado en obtener con éxito

una nueva composición terapéutica para el tratamiento del cáncer que comprenda un anticuerpo contra el receptor del Factor de Crecimiento Epidérmico (EGFR) y uno o más interferones tipo 1, por lo que evidentemente carecería de altura o nivel inventivo. Además, la obtención de la composición terapéutica propuesta como solución al problema técnico planteado a partir de dicha combinación también carece evidentemente de altura inventiva.

2.6. Por lo tanto, este documento objeto de oposición a la luz del documento D1 en combinación con el documento D2, carece de ALTURA INVENTIVA.

2.7. En consecuencia y de acuerdo a lo descrito anteriormente, se deriva que la solicitud colombiana 09-30399 no puede pretender un monopolio por patente toda vez que lo que pretende no cumple con dos de los tres requisitos necesarios para su protección.

2.8. Que por consiguiente, respecto a la solicitud pretendida se tiene que:

2.8.1. CARENCIA DE ALTURA INVENTIVA. Según la Decisión 486 de la Comunidad Andina de Naciones, Artículo 18.- *“Se considerará que una invención tiene nivel inventivo, si para una persona del oficio normalmente versada en la materia técnica correspondiente, esa invención no hubiese resultado obvia ni se hubiese derivado de manera evidente del estado de la técnica.”*. A la fecha de prioridad, 29 de septiembre de 2006, a partir del documento D2 en combinación con D1, puede llegar de manera evidente a composiciones terapéuticas para potenciar el efecto de la terapia con anticuerpos contra el receptor del factor de crecimiento epidérmico, sobre el cual busca protección en el capítulo reivindicatorio.

2.8.2. CARENCIA DE APLICACIÓN INDUSTRIAL La Decisión 486 de la CAN consagra en su artículo 14 lo siguiente: *“Los países miembros otorgarán patentes para las invenciones, sean de producto o procedimiento, en todos los campos de la*

tecnología, siempre que sean nuevas, tengan nivel inventivo y sean susceptibles de aplicación industrial". La presente solicitud carece de aplicación Industrial ya que como se demostró anteriormente, algunas cláusulas reclaman el método terapéutico y estos no son aplicables industrialmente porque primero no se obtiene un producto o proceso; y segundo no es reproducible o repetible a escala industrial. Por lo tanto, el objeto de la presente invención referido a los usos y métodos terapéuticos, no cumplen con el requisito de la aplicación industrial.

2.9. Dado lo anterior, es posible deducir que la solicitud y lo reivindicado no es objeto de patentabilidad pues es contrario a lo que dicta la Decisión 486 de la Comunidad Andina de Naciones, Artículo 14.- *"Los Países Miembros otorgarán patentes para las invenciones, sean de producto o de procedimiento, en todos los campos de la tecnología, siempre que sean nuevas, tengan nivel inventivo y sean susceptibles de aplicación industrial."* La solicitud pretendida no cumple con dos de los tres requisitos de patentabilidad.

2.10. Por todo lo anterior, atentamente solicito a usted que se sirva declarar fundada "COMPOSICIONES TERAPEUTICAS PARA POTENCIAR EL EFECTO DE LA TERAPIA CON ANTICUERPOS CONTRA EL RECEPTOR DEL FACTOR DE CRECIMIENTO EPIDERMICO", cuyo titular es **CENTRO DE INMUNOLOGIA MOLECULAR**, solicitud Colombiana que apareció publicada en la Gaceta de la Propiedad Industrial N° 616 del 20 de mayo de 2010.

3. PRUEBAS

De conformidad con lo dispuesto por las normas vigentes, solicito a usted tener como prueba en este asunto los siguientes:

- D1: WO02089842 publicada el 14 de noviembre de 2002.
- D2: US2006134064 publicada el 22 de junio de 2006.

4. FUNDAMENTO DE DERECHO

Fundamento esta observación en las normas legales siguientes:

- 4.1. Numeral 1 del artículo 586 del Código de Comercio.
- 4.2. Artículo 42 de la Decisión 486 de la Comisión del Acuerdo de Cartagena.
- 4.3. En las demás normas concordantes.

5. ANEXOS

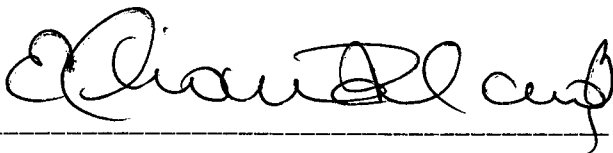
Para los efectos correspondientes anexo al presente escrito los siguientes documentos:

1. Recibo de caja del respectivo pago de tasa.
2. Copias de los documentos:
 - D1: WO02089842 publicada el 14 de noviembre de 2002.
 - D2: US2006134064 publicada el 22 de junio de 2006.

6. NOTIFICACIONES

Para efectos de notificaciones que por disposición del artículo 50 del Decreto 01 de 1984 debe hacer su Despacho, informo que mi representada y la suscrita recibiremos notificaciones en la Secretaría de su Despacho o en mis oficinas situadas en la Carrera 13 N° 119-95, Of. 104 de esta ciudad de Bogotá.

Señor Superintendente,



ELIANA ISAURA MORENO BOHORQUEZ

C.C. 51'975.664 de Bogotá

T.P.A. 83823 del C.S. de la J.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 10 - 58,548
FECHA : JULIO 16 DE 2010

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 09-030399.-00003-0000

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Tra. 11 PATENTEIYII Eve: 379 FASENACIONALII
Act. 400 OPOSIOBSERVTER Folios: 87

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
LAB. PROCAPS	CONSIGNACION	BANCO DE BOGOTA	062754387	132583942	09/07/2010	2,065,000.00
LAB. PROCAPS	CONSIGNACION	BANCO DE BOGOTA	062754387	381044985	14/07/2010	2,065,000.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-04	PRESENTACION DE OBSERVACIONES	
12		MARCAS Y LEMAS COMERCIALES	295,000.00
		TOTAL :	\$ 295,000.00

SON: DOSCIENTOS NOVENTA Y CINCO MIL PESOS

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

ANEXO 1

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
14 November 2002 (14.11.2002)

PCT

(10) International Publication Number
WO 02/089842 A1

(51) International Patent Classification⁷: **A61K 39/395**,
31/00, C07K 16/28, 16/46, A61P 35/00, A61K 38/00 //
(A61K 39/395, 31:00) (A61K 39/395, 38:00)

(21) International Application Number: PCT/EP02/04404

(22) International Filing Date: 22 April 2002 (22.04.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
01111049.1 8 May 2001 (08.05.2001) EP

(71) Applicant (for all designated States except US): **MERCK
PATENT GMBH** [DE/DE]; Frankfurter Strasse 250,
64293 Darmstadt (DE).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **ROSEN, Oliver**
[DE/DE]; Lucasweg 23, 64287 Darmstadt (DE).
HARSTRICK, Andreas [DE/DE]; Raibachertal 48,
64823 Gross-Umstadt (DE).

(74) Common Representative: **MERCK PATENT GMBH**;
Frankfurter Strasse 250, 64293 Darmstadt (DE).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG,
SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ,
VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR,
GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent
(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,
NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.



WO 02/089842 A1

(54) Title: COMBINATION THERAPY USING ANTI-EGFR ANTIBODIES AND ANTI-HORMONAL AGENTS

(57) Abstract: The invention relates to a combination therapy for the treatment of tumors and tumor metastases, preferably breast and prostate tumors, comprising administration of anti-EGFR (Her1) antibodies and anti-hormonal agents, optionally together with cytotoxic / chemotherapeutic agent. The method and the pharmaceutical compositions comprising said agents can result in a synergistic potentiation of the tumor cell proliferation inhibition effect of each individual therapeutic agent, yielding more effective treatment than found by administering an individual component alone.

COMBINATION THERAPY USING ANTI-EGFR ANTIBODIES AND ANTI-HORMONAL AGENTS

TECHNICAL FIELD OF THE INVENTION:

- 5 The invention relates to a combination therapy for the treatment of tumors and tumor metastases, preferably breast and prostate tumors, comprising administration of anti-EGFR (Her1) antibodies and anti-hormonal agents, optionally together with cytotoxic / chemotherapeutic agents. The method and the pharmaceutical compositions comprising said agents can result in a synergistic
- 10 potentiation of the tumor cell proliferation inhibition effect of each individual therapeutic agent, yielding more effective treatment than found by administering an individual component alone.

BACKGROUND OF THE INVENTION:

- 15 Tyrosine kinases are a class of enzymes that catalyze the transfer of the terminal phosphate of adenosine triphosphate to tyrosine residues in protein substrates. Tyrosine kinases are believed, by way of substrate phosphorylation, to play critical roles in signal transduction for a number of cell functions. Though the exact mechanisms of signal transduction is still unclear, tyrosine kinases have
- 20 been shown to be important contributing factors in cell proliferation, carcinogenesis and cell differentiation.
- Tyrosine kinases can be categorized as receptor type or non-receptor type. Both receptor-type and non-receptor type tyrosine kinases are implicated in cellular signaling pathways leading to numerous pathogenic conditions, including cancer,
- 25 psoriasis and hyperimmune responses. Many tyrosine kinases are involved in cell growth as well as in angiogenesis.

- The non-receptor type of tyrosine kinases is also comprised of numerous subfamilies, including Src, Frk, Btk, Csk, Abl, Zap70, Fes/Fps, Fak, Jak, Ack, and
- 30 LIMK. Each of these subfamilies is further sub-divided into varying receptors. For example, the Src subfamily is one of the largest and includes Src, Yes, Fyn, Lyn, Lck, Blk, Hck, Fgr, and Yrk. The Src subfamily of enzymes has been linked to oncogenesis. For a more detailed discussion of the non-receptor type of tyrosine kinases, see Bolen *Oncogene*, 8:2025-2031 (1993).

Receptor type tyrosine kinases have an extracellular, a transmembrane, and an intracellular portion, while non-receptor type tyrosine kinases are wholly intracellular. Receptor-linked tyrosine kinases are transmembrane proteins that contain an extracellular ligand binding domain, a transmembrane sequence, and a cytoplasmic tyrosine kinase domain. The receptor-type tyrosine kinases are comprised of a large number of transmembrane receptors with diverse biological activity. In fact, different subfamilies of receptor-type tyrosine kinases have been identified. Implicated tyrosine kinases include fibroblast growth factor (FGF) receptors, epidermal growth factor (EGF) receptors of the ErbB major class family, and platelet-derived growth factor (PDGF) receptors. Also implicated are nerve growth Factor (NGF) receptors, brain-derived neurotrophic Factor (BDNF) receptors, and neurotrophin-3 (NT-3) receptors, and neurotrophin-4 (NT-4) receptors.

One receptor type tyrosine kinase subfamily, designated as HER or ErbB subfamily, is comprised of EGFR (ErbB1), HER2 (ErbB2 or p185neu), HER3 (ErbB3), and HER4 (ErbB4 or tyro2). Ligands of this subfamily of receptors include epithelial growth factor (EGF), TGF- α , amphiregulin, HB-EGF, betacellulin and heregulin. The PDGF subfamily includes the FLK family which is comprised of the kinase insert domain receptor (KDR).

EGFR, encoded by the erbB1 gene, has been causally implicated in human malignancy. In particular, increased expression of EGFR has been observed in breast, bladder, lung, head, neck and stomach cancer as well as glioblastomas. Increased EGFR receptor expression is often associated with increased production of the EGFR ligand, transforming growth factor alpha (TGF- α), by the same tumor cells resulting in receptor activation by an autocrine stimulatory pathway (Baselga and Mendelsohn, *Pharmac. Ther.* 64:127-154 (1994)).

The EGF receptor is a transmembrane glycoprotein which has a molecular weight of 170.000, and is found on many epithelial cell types. It is activated by at least three ligands, EGF, TGF- α (transforming growth factor alpha) and amphiregulin. Both epidermal growth factor (EGF) and transforming growth factor-alpha (TGF- α) have been demonstrated to bind to EGF receptor and to

lead to cellular proliferation and tumor growth. These growth factors do not bind to HER2 (Ulrich and Schlesinger, 1990, Cell 61, 203). In contrary to several families of growth factors, which induce receptor dimerization by virtue of their dimeric nature (e.g. PDGF) monomeric growth factors, such as EGF, contain two binding sites for their receptors and, therefore, can cross-link two neighboring EGF receptors (Lemmon et al., 1997, EMBO J. 16, 281). Receptor dimerization is essential for stimulating of the intrinsic catalytic activity and for the auto-phosphorylation of growth factor receptors. It should be remarked that receptor protein tyrosine kinases (PTKs) are able to undergo both homo- and heterodimerization.

It has been demonstrated that anti-EGF receptor antibodies while blocking EGF and TGF- α binding to the receptor appear to inhibit tumor cell proliferation. In view of these findings, a number of murine and rat monoclonal antibodies against EGF receptor have been developed and tested for their ability inhibit the growth of tumor cells in vitro and in vivo (Modjtahedi and Dean, 1994, *J. Oncology* 4, 277). Humanized monoclonal antibody 425 (hMAb 425, US 5,558,864; EP 0531 472) and chimeric monoclonal antibody 225 (cMAb 225, US 4,943,533 and EP 0359 282), both directed to the EGF receptor, have shown their efficacy in clinical trials. The C225 antibody was demonstrated to inhibit EGF-mediated tumor cell growth in vitro and inhibit human tumor formation in vivo in nude mice. The antibody, moreover, appeared to act, above all, in synergy with certain chemotherapeutic agents (i.e., doxorubicin, adriamycin, taxol, and cisplatin) to eradicate human tumors in vivo in xenograft mouse models. Ye et al. (1999, *Oncogene* 18, 731) have reported that human ovarian cancer cells can be treated successfully with a combination of both cMAb 225 and humanized MAb 4D5 which is directed to the HER2 receptor.

The second member of the ErbB family, HER2 (ErbB2 or p185 neu), was originally identified as the product of the transforming gene from neuroblastomas of chemically treated rats. The activated form of the neu proto-oncogene results from a point mutation (valine to glutamic acid) in the transmembrane region of the encoded protein. Amplification of the human homolog of neu is observed in breast and ovarian cancers and correlates with a poor prognosis (Slamon *et al.*,

Science, 235: 177-182 (1987); Slamon *et al.*, *Science*, 244:707-712 (1989); US 4,968,603). ErbB2 (HER2) has a molecular weight of about 185,000, with considerable homology to the EGF receptor (HER1), although a specific ligand for HER2 has not yet been clearly identified so far.

5 The antibody 4D5 directed to the HER2 receptor, was further found to sensitize ErbB2-overexpressing breast tumor cell lines to the cytotoxic effects of TNF α (US 5,677,171). A recombinant humanized version of the murine anti-ErbB2 antibody 4D5 (huMAb4D5-8, rhuMAb HER2 or HERCEPTIN®; US 5,821,337) is clinically active in patients with ErbB2-overexpressing metastatic breast cancers that have
10 received extensive prior anti-cancer therapy (Baselga *et al.*, *J. Clin. Oncol.* 14:737-744 (1996)). HERCEPTIN® received marketing approval in 1998 for the treatment of patients with metastatic breast cancer whose tumors overexpress the ErbB2 protein.

15 Prostate and breast cancer are the most frequently diagnosed cancers in men / women and are responsible for more than several hundred thousands deaths worldwide annually. Early stage, organ-confined, prostate and breast cancer is often managed with surgery or radiation therapy until the patient dies from unrelated causes. Carcinomas such as breast cancer, colon cancer
20 and adenocarcinoma are characterized by rapid cell division. Consequently, these cancers are amenable to treatment with chemotherapeutic agents that inhibit rapid cell division. In contrast, prostate cancer is not characterized by rapid cell division. Therefore, conventional chemotherapeutic agents generally display low efficacy against prostatic carcinomas. Prostatic carcinomas are often
25 sensitive to hormonal manipulation. Currently approved treatment of prostate cancer includes surgical castration, chemical castration, or a combination of surgical and chemical castration.

Removal of the testes, the primary testosterone producing organ, reduces the
30 levels of circulating androgens, to less than 5% of normal levels. This reduction in androgen levels inhibits prostate tumor growth. Although the anti-tumor effects of surgical castration are direct, the anti-tumor effects can be temporary. Surgical castration often leads to clonal selection of androgen-independent prostate tumor

cells. This results in re-growth of the prostate tumor in a form that proliferates without testosterone or DHT Stimulation (Isaacs et al. (1981) *Cancer Res.* 41:5070-5075; Crawford et al. (1989) *IV. Eng. J. Med.* 321:419-424). Chemical castration (also called medical castration) is often substituted for surgical
5 castration, as an initial treatment.

Prostate and breast cancer share a unique feature which is that in the majority they are stimulated in growth by steroid sex hormones (estrogens for breast cancer and androgens for prostate cancer, respectively). The steroid sex hormones deliver their stimulating signals by binding to specific receptors.

10 Estrogene and androgene receptors can be found on approximately 75% of breast cancer cells and nearly 100% of prostate cancer cells and are members of the nuclear hormone receptor superfamily which includes the receptors for steroid hormones, thyroid hormones, lipophilic vitamins such as vitamins A and D, and the orphan receptors, which have a structure consistent with other superfamily
15 members but have no identified ligands (Evans (1988) *Science* 240:889-895). The receptors regulate gene expression by interacting with specific DNA sequences in the promoters of target genes (Glass (1994) *Endocr. Rev.* 15:391-407).

Nuclear receptors are grouped into two subfamilies: the thyroid/retinoic
20 acid/vitamin D receptor (TRV) family and the steroid receptor (R_s) family. Steroid hormone receptors bind to their respective HREs in a ligand-dependent manner whereas some receptors such as the thyroid hormone receptor (T3R) and retinoic acid receptor (RAR/RXR) bind to their response elements in a ligand-independent manner. Steroid receptors play a role in normal health and in a spectrum of
25 disease states, including cancer, inflammation, endocrine disorders, and oral contraception. The natural steroid hormones produced by endocrine glands bind to steroid hormone receptors in target organs.

The natural steroid hormones include estrogens, progestins, androgens,
30 glucocorticoids and mineralocorticoids. These hormones are defined as agonists, and hormone-receptor complexes modulate specific gene transcription by either increasing or decreasing transcription rate.

1 Steroid agonists have pleiotropic physiological actions in a number of tissues, for example, estradiol and progesterone regulate gene transcription in the kidney, ovary, cervix, uterus, bone, skin, breast, heart, pituitary and brain. Hormones of the steroid receptor subfamily are used to treat many disorders and are used in
5 healthy people for oral contraception and hormone replacement therapy, among others.

It is often medically desirable to block the actions of steroid hormone agonists. For this reason, researchers have synthesized steroid receptor antagonists that
10 are used in breast, endometrial and prostate cancer treatment as agents to prevent cancer development or block abnormal growths, and as contraceptive agents. These antagonists are ligands also to the steroid receptors, but in general, they have effects that are opposite to the ones produced by the agonists.

15 The actions of steroid receptor antagonist are complex. They often have dual agonist/antagonist effects. For example, an antagonist may have partially the biological activity of an agonist; thus, the antagonist may block the activity of the agonist, resulting in substantially decreased agonist activity. Antagonists may
20 also have the desired antagonist effect in one tissue (for example, the breast), but may have an agonist effect in another tissue (for example, the Uterus). The agonist effect of an antagonist may or may not be an unwanted side-effect. Similarly, in cancer treatment, an antagonist ligand may initially have the desired inhibitory effect on the tumor, but with time, the ligand switches to an agonist-like
25 effect and the cancer then resumes growing.

A typical example is LHRH (luteinizing hormone-releasing hormone). LHRH active compounds administered in individual single higher doses stimulate the hormone production (agonist action), whereas continuous small doses of said compounds act as antagonists inhibiting the hormone releasing effect.

30

Angiogenesis, also referred to as neovascularization, is a process of tissue vascularization that involves the growth of new developing blood vessels into a tissue. The process is mediated by the infiltration of endothelial cells and smooth

muscle cells. The process is believed to proceed in any one of three ways: (1) The vessels can sprout from pre-existing vessels; (2) De novo development of vessels can arise from precursor cells (vasculogenesis); or (3) Existing small vessels can enlarge in diameter (Blood et al., 1990, Bioch. Biophys. Acta 1032, 89). Vascular endothelial cells are known to contain at least five RGD-dependent integrins, including the vitronectin receptor ($\alpha_v\beta_3$ or $\alpha_v\beta_5$), the collagen Types I and IV receptor, the laminin receptor, the fibronectin/laminin/collagen receptor and the fibronectin receptor (Davis et al., 1993, J. Cell. Biochem. 51, 206). The smooth muscle cell is known to contain at least six RGD-dependent integrins, including $\alpha_v\beta_3$ $\alpha_v\beta_5$.

Angiogenesis is an important process in neonatal growth, but is also important in wound healing and in the pathogenesis of a large variety of clinically important diseases including tissue inflammation, arthritis, psoriasis, cancer, diabetic retinopathy, macular degeneration and other neovascular eye diseases. These clinical entities associated with angiogenesis are referred to as angiogenic diseases (Folkman et al., 1987, Science 235, 442).

Inhibition of cell adhesion in vitro using monoclonal antibodies immunospecific for various integrin α or β subunits have implicated the vitronectin receptor $\alpha_v\beta_3$ in cell adhesion of a variety of cell types including microvascular endothelial cells (Davis et al., 1993, J. Cell. Biol. 51, 206).

Integrins are a class of cellular receptors known to bind extracellular matrix proteins, and therefore mediate cell-cell and cell-extracellular matrix interactions, referred generally to as cell adhesion events. The integrin receptors constitute a family of proteins with shared structural characteristics of noncovalent heterodimeric glycoprotein complexes formed of α and β subunits. The vitronectin receptor, named for its original characteristic of preferential binding to vitronectin, is now known to refer to three different integrins, designated $\alpha_v\beta_1$, $\alpha_v\beta_3$ and $\alpha_v\beta_5$. $\alpha_v\beta_1$ binds fibronectin and vitronectin. $\alpha_v\beta_3$ binds a large variety of ligands, including fibrin, fibrinogen, laminin, thrombospondin, vitronectin and von Willebrand's factor. $\alpha_v\beta_5$ binds vitronectin. It is clear that there are different integrins with different biological functions as well as different integrins and subunits having shared biological specificity. One important recognition site in a ligand for many integrins is the arginine-glycine-aspartic acid (RGD) tripeptide

sequence. RGD is found in all of the ligands identified above for the vitronectin receptor integrins.

This RGD recognition site can be mimicked by linear and cyclic (poly)peptides that contain the RGD sequence. Such RGD peptides are known to be inhibitors or antagonists, respectively, of integrin function. It is important to note, however, that depending upon the sequence and structure of the RGD peptide, the specificity of the inhibition can be altered to target specific integrins. Various RGD polypeptides of varying integrin specificity have been described, for example, by Cheresch, et al., 1989, Cell 58, 945, Aumailley et al., 1991, FEBS Letts. 291, 50, and in numerous patent applications and patents (e.g. US patents 4,517,686, 4,578,079, 4,589,881, 4,614,517, 4,661,111, 4,792,525; EP 0770 622).

The generation of new blood vessels, or angiogenesis, plays a key role in the growth of malignant disease and has generated much interest in developing agents that inhibit angiogenesis (see, for example, Holmgren et al., 1995, Nature Medicine 1, 149; Folkman, 1995, Nature Medicine 1, 27; O'Reilly et. al., 1994, Cell 79, 315). The use of $\alpha_v\beta_3$ integrin antagonists to inhibit angiogenesis is known in methods to inhibit solid tumor growth by reduction of the blood supply to the solid tumor (see, for example, US 5,753,230 and US 5,766,591, which describe the use of $\alpha_v\beta_3$ antagonists such as synthetic polypeptides, monoclonal antibodies and mimetics of $\alpha_v\beta_3$ that bind to the $\alpha_v\beta_3$ receptor and inhibit angiogenesis). Methods and compositions for inhibiting $\alpha_v\beta_5$ mediated angiogenesis of tissues using antagonists of the vitronectin receptor $\alpha_v\beta_5$ are disclosed in WO 97/45447.

Angiogenesis is characterized by invasion, migration and proliferation of endothelial cells, processes that depend on cell interactions with extracellular matrix components. In this context, the integrin cell-matrix receptors mediate cell spreading and migration. The endothelial adhesion receptors of integrin $\alpha_v\beta_3$ was shown to be a key player by providing a vasculature-specific target for anti-angiogenic treatment strategies (Brooks et al., 1994, Science 264, 569; Friedlander et. al., 1995, Science 270). The requirement for vascular integrin $\alpha_v\beta_3$ in angiogenesis was demonstrated by several in vivo models where the generation of new blood vessels by transplanted human tumors was entirely

inhibited either by systemic administration of peptide antagonists of integrin $\alpha_v\beta_3$ and $\alpha_v\beta_5$, as indicated above, or, alternatively, by anti- $\alpha_v\beta_3$ antibody LM609 (Brooks et al., 1994, Cell 79, 1157; ATCC HB 9537). This antibody blocks the $\alpha_v\beta_3$ integrin receptor the activation of which by its natural ligands promotes
5 apoptosis of the proliferative angiogenic vascular cells and thereby disrupts the maturation of newly forming blood vessels, an event essential for the proliferation of tumors. Nevertheless, it was recently reported, that melanoma cells could form web-like patterns of blood vessels even in the absence of endothelial cells (1999, Science 285, 14), implying that tumors might be able to circumvent some anti-
10 angiogenic drugs which are only effective in the presence of endothelial tissue.

Numerous molecules stimulate endothelial proliferation, migration and assembly, including VEGF, Ang1 and bFGF, and are vital survival factors. VEGF (Vascular Endothelial Growth Factor) has been identified as a selective angiogenic growth
15 factor that can stimulate endothelial cell mitogenesis. VEGF, in particular, is thought to be a major mediator of angiogenesis in a primary tumor and in ischemic ocular diseases. VEGF is a homodimer (MW : 46.000) that is an endothelial cell-specific angiogenic (Ferrara et al., 1992, Endocrin. Rev., 13, 18) and vasopermeable factor (Senger et al., 1986, Cancer Res., 46:5629) that binds
20 to high-affinity membrane-bound receptors with tyrosine kinase activity (Jakeman et al., 1992, J. Clin. Invest., 89, 244). Human tumor biopsies exhibit enhanced expression of VEGF mRNAs by malignant cells and VEGF receptor mRNAs in adjacent endothelial cells. VEGF expression appears to be greatest in regions of tumors adjacent to vascular areas of necrosis. (for review see Thomas et al.,
25 1996, J. Biol. Chem. 271(2), 603; Folkman, 1995, Nature Medicine 1, 27). WO 97/45447 has implicated the $\alpha_v\beta_5$ integrin in neovascularization, particularly, that induced by VEGF, EGF and TGF- α , and discloses that $\alpha_v\beta_5$ antagonist can inhibit VEGF promoted angiogenesis. Effective anti-tumor therapies may also utilize targeting VEGF receptor for inhibition of angiogenesis using monoclonal
30 antibodies. (Witte et al., 1998, Cancer Metastasis Rev. 17(2), 155). MAb DC-101 is known to inhibit angiogenesis of tumor cells.

The present invention describes now that antibodies specifically directed to EGF receptor (ErbB1, Her1) are more effective in killing or shrinking tumor tissue of especially prostate and breast cancer when administered together with anti-hormonal agents, especially inhibitors of the nuclear hormone receptor family.

5 Further co-administration with anti-angiogenic agents and / or cytotoxic agents may improve the positive and synergistic effect of said combination therapy.

SUMMARY OF THE INVENTION

The present inventions describes for the first time the new concept in tumor

10 therapy to administer to an individual an agent that blocks or inhibits the EGF receptor together with an anti-hormonal agent. Optionally the composition according to this invention comprises further therapeutically active compounds, preferably selected from the group consisting of cytotoxic agents, chemotherapeutic agents and inhibitors or antagonists of the ErbB receptor

15 tyrosine kinase family or inhibitors or antagonist of angiogenesis .

Thus, the invention relates to pharmaceutical compositions comprising as preferred anti-EGFR agent an anti-EGFR antibody and as anti-hormonal agent an inhibitor or antagonist of the nuclear receptor, preferably an steroid receptor.

20 According to this invention said therapeutically active agents may also be provided by means of a pharmaceutical kit comprising a package comprising one or more anti-EGFR antibody, one or more anti-hormonal agents, and, optionally, one or more cytotoxic / chemotherapeutic agents, anti-ErbB agents, anti-angiogenic agents in single packages or in separate containers. The therapy with

25 this combinations may include optionally treatment with radiation.

However, the invention relates, furthermore, to a combination therapy comprising the administration of only one (fusion) molecule, having anti-EGFR activity and anti-hormonal activity, optionally together with one or more cytotoxic /

30 chemotherapeutic agents. An example is an anti-EGFR antibody, such as h425 or c225 as described above and below, which is fused at the C-terminal of its Fc portion to an anti-hormonal agent by known recombinant or chemical methods .

A further example is a bispecific antibody, wherein one specificity is directed to an nuclear hormone receptor and the other one is directed to the EGF receptor.

Principally, the administration can be accompanied by radiation therapy, wherein
5 radiation treatment can be done substantially concurrently or before or after the
drug administration. The administration of the different agents of the combination
therapy according to the invention can also be achieved substantially
concurrently or sequentially. Tumors, bearing receptors on their cell surfaces
involved in the development of the blood vessels of the tumor, may be
10 successfully treated by the combination therapy of this invention.

It is known that tumors elicit alternative routes for their development and growth.
If one route is blocked they often have the capability to switch to another route by
expressing and using other receptors and signaling pathways. Therefore, the
15 pharmaceutical combinations of the present invention may block several of such
possible development strategies of the tumor and provide consequently various
benefits. The combinations according to the present invention are useful in
treating and preventing tumors, tumor-like and neoplasia disorders and tumor
metastases, which develop and grow by activation of their relevant hormone
20 receptors which are present on the surface of the tumor cells. Preferably, the
different combined agents of the present invention are administered in
combination at a low dose, that is, at a dose lower than has been
conventionally used in clinical situations. A benefit of lowering the dose of the
compounds, compositions, agents and therapies of the present invention
25 administered to an individual includes a decrease in the incidence of adverse
effects associated with higher dosages. For example, by the lowering the
dosage of an agent described above and below, a reduction in the frequency and
the severity of nausea and vomiting will result when compared to that observed at
higher dosages. By lowering the incidence of adverse effects, an improvement in
30 the quality of life of a cancer patient is contemplated. Further benefits of lowering
the incidence of adverse effects include an improvement in patient compliance,
a reduction in the number of hospitalizations needed for the treatment of adverse
effects, and a reduction in the administration of analgesic agents needed to treat

pain associated with the adverse effects. Alternatively, the methods and combination of the present invention can also maximize the therapeutic effect at higher doses.

The combinations according to the inventions show an astonishing synergetic effect. In administering the combination of drugs real tumor shrinking and
5 disintegration could be observed during clinical studies while no significant adverse drug reactions were detectable.

In detail the invention refers to:

- 10 • a pharmaceutical composition comprising in an therapeutically effective amount at least (i) one anti-EGFR antibody or an immunotherapeutically effective fragment thereof and (ii) one anti-hormonal agent, optionally together with a pharmaceutically acceptable carrier, excipient or diluent;
- a corresponding pharmaceutical composition, wherein said anti-EGFR
15 antibody or said immunotherapeutically effective fragment thereof is murine, chimeric or humanized Mab 425 (h425) or chimeric Mab 225 (c225) inhibitor/antagonist;
- a corresponding pharmaceutical composition, wherein said anti-hormonal agent is an inhibitor of the nuclear hormone receptor family;
- 20 • a corresponding pharmaceutical composition, wherein said anti-hormonal agent is an steroid receptor inhibitor/antagonist;
- a corresponding pharmaceutical composition comprising additionally an anti-angiogenic agent;
- a corresponding pharmaceutical composition further comprising a cytotoxic
25 and / or chemotherapeutic agent;
- a corresponding pharmaceutical composition comprising additionally a further an anti-HER2 antibody or an immunotherapeutically active fragment thereof;
- a pharmaceutical composition comprising an antibody having an anti-EGFR activity and an anti-nuclear hormone receptor activity, optionally together with
30 a pharmaceutically acceptable carrier, excipient or diluent.;
- a corresponding pharmaceutical, wherein said antibody is a bispecific antibody;

- a pharmaceutical kit comprising a package comprising at least
(i) one anti-EGFR antibody or an immunotherapeutically effective fragment thereof, and (ii) one anti-hormonal agent, and optionally (iii) a cytotoxic and/or chemotherapeutic agent;
- 5 • a corresponding pharmaceutical kit comprising (i) monoclonal antibody h425, and (ii) a steroid receptor antagonist;
- a corresponding pharmaceutical kit comprising (i) monoclonal antibody h425, and (ii) a LHRH antagonist;
- a corresponding pharmaceutical kit, wherein said pharmaceutically active
10 agents are provided in separate containers in said package;
- the use of a pharmaceutical composition or a pharmaceutical kit as defined above and below for the manufacture of a medicament or a composition of medicaments to treat tumors and tumor metastases preferably for the treatment of breast and prostate cancer;
- 15 • the corresponding use for the treatment of steroid-independent breast and prostate cancer;
- a method for treating tumors or tumor metastases in an individual comprising administering to said individual simultaneously or sequentially a therapeutically effective amount of (i) an anti-EGFR antibody, and (ii) an anti-
20 hormonal agent;
- a corresponding method, wherein said anti-EGFR antibody is monoclonal antibody h425 or c225 and said anti-hormonal agent is an steroid receptor antagonist; and
- a corresponding method comprising administering additionally to said
25 individual a therapeutically effective amount of a cytotoxic and/or chemotherapeutic agent or an anti-angiogenic agent or another anti-ErbB receptor antibody.

DETAILED DESCRIPTION OF THE INVENTION

- 30 If not otherwise pointed out the terms and phrases used in this invention have the meanings and definitions as given below. Moreover, these definitions and meanings describe the invention in more detail, preferred embodiments included.

"**Biological molecules**" include natural or synthetic molecules having, as a rule, a molecular weight greater than approximately 300, and are preferably poly- and oligosaccharides, oligo- and polypeptides, proteins, peptides, poly- and oligonucleotides as well as their glycosylated lipid derivatives. Most typically, biological molecules include immunotherapeutic agents, above all antibodies or fragments thereof, or functional derivatives of these antibodies or fragments including fusion proteins.

A "**receptor**" or "**receptor molecule**" is a soluble or membrane bound / associated protein or glycoprotein comprising one or more domains to which a ligand binds to form a receptor-ligand complex. By binding the ligand, which may be an agonist or an antagonist the receptor is activated or inactivated and may initiate or block pathway signaling.

By "**ligand**" or "**receptor ligand**" is meant a natural or synthetic compound which binds a receptor molecule to form a receptor-ligand complex. The term ligand includes agonists, antagonists, and compounds with partial agonist/antagonist action.

An "**agonist**" or "**receptor agonist**" is a natural or synthetic compound which binds the receptor to form a receptor-agonist complex by activating said receptor and receptor-agonist complex, respectively, initiating a pathway signaling and further biological processes.

By "**antagonist**" or "**receptor antagonist**" is meant a natural or synthetic compound that has a biological effect opposite to that of an agonist. An antagonist binds the receptor and blocks the action of a receptor agonist by competing with the agonist for receptor. An antagonist is defined by its ability to block the actions of an agonist. A receptor antagonist may be also an antibody or an immunotherapeutically effective fragment thereof. Preferred antagonists according to the present invention are cited and discussed below.

The term "**therapeutically effective**" or "**therapeutically effective amount**" refers to an amount of a drug effective to treat a disease or disorder in a mammal. In the case of cancer, the therapeutically effective amount of the drug may reduce the number of cancer cells; reduce the tumor size; inhibit (i.e., slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (i.e., slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. To the extent the drug may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic. For cancer therapy, efficacy can, for example, be measured by assessing the time to disease progression (TTP) and/or determining the response rate (RR).

The term "**immunotherapeutically effective**" refers to biological molecules which cause an immune response in a mammal. More specifically, the term refers to molecules which may recognize and bind an antigen. Typically, antibodies, antibody fragments and antibody fusion proteins comprising their antigen binding sites (complementary determining regions, CDRs) are immunotherapeutically effective.

An "**anti-angiogenic agent**" refers to a natural or synthetic compound which blocks, or interferes with to some degree, the development of blood vessels. The anti-angiogenic molecule may, for instance, be a biological molecule that binds to and blocks an angiogenic growth factor or growth factor receptor. The preferred anti-angiogenic molecule herein binds to an receptor, preferably to an integrin receptor or to VEGF receptor. The term includes according to the invention also a prodrug of said angiogenic agent.

There are a lot of molecules having different structure and origin which elicit anti-angiogenic properties. Most relevant classes of angiogenesis inhibiting or blocking agents which are suitable in this invention, are, for example:

- (i) anti-mitotics such as flurouracil, mytomycin-C, taxol;
- (ii) estrogen metabolites such as 2-methoxyestradiol;
- (iii) matrix metalloproteinase (MMP) inhibitors, which inhibit zinc

metalloproteinases (metalloproteases) (e.g. betimastat, BB16, TIMPs, minocycline, GM6001, or those described in "Inhibition of Matrix Metalloproteinases: Therapeutic Applications" (Golub, Annals of the New York Academy of Science, Vol. 878a; Greenwald, Zucker (Eds.), 1999);

- 5 (iv) anti-angiogenic multi-functional agents and factors such as IFN α (US 4,530,901; US 4,503,035; 5,231,176); angiostatin and plasminogen fragments (e.g. kringle 1-4, kringle 5, kringle 1-3 (O'Reilly, M. S. et al., *Cell (Cambridge, Mass.)* 79(2): 315-328, 1994; Cao et al., *J. Biol. Chem.* 271: 29461-29467, 1996; Cao et al., *J. Biol Chem* 272: 22924 -22928, 1997);
- 10 endostatin (O'Reilly, M. S. et al., *Cell* 88(2), 277, 1997 and WO 97/15666), thrombospondin (TSP-1; Frazier, 1991, *Curr Opin Cell Biol* 3(5): 792); platelet factor 4 (PF4);
- (v) plasminogen activator/urokinase inhibitors;
- (vi) urokinase receptor antagonists;
- 15 (vii) heparinases;
- (viii) fumagillin analogs such as TNP-470;
- (ix) tyrosine kinase inhibitors such as SUI 01 (many of the above and below - mentioned ErbB receptor antagonists (EGFR / HER2 antagonists) are also tyrosine kinase inhibitors, and may show, therefore anti-EGF receptor blocking
- 20 activity which results in inhibiting tumor growth, as well as anti-angiogenic activity which results in inhibiting the development of blood vessels and endothelial cells, respectively);
- (x) suramin and suramin analogs;
- (xi) angiostatic steroids;
- 25 (xii) VEGF and bFGF antagonists;
- (xiii) VEGF receptor antagonists such as anti-VEGF receptor antibodies (DC-101);
- (xiv) flk-1 and flt-1 antagonists;
- (xv) cyclooxygenase-II inhibitors such as COX-II;
- 30 (xvi) integrin antagonists and integrin receptor antagonists such as α_v antagonists and α_v receptor antagonists, for example, anti- α_v receptor antibodies and RGD peptides. Integrin (receptor) antagonists are preferred according to this invention.

The term "**integrin antagonists / inhibitors**" or "**integrin receptor antagonists / inhibitors**" refers to a natural or synthetic molecule that blocks and inhibit an integrin receptor. In some cases, the term includes antagonists directed to the ligands of said integrin receptors (such as for $\alpha_v\beta_3$: vitronectin, fibrin, fibrinogen, von Willebrand's factor, thrombospondin, laminin; for $\alpha_v\beta_5$: vitronectin; for $\alpha_v\beta_1$: fibronectin and vitronectin; for $\alpha_v\beta_6$: fibronectin).

Antagonists directed to the integrin receptors are preferred according to the invention. Integrin (receptor) antagonists may be natural or synthetic peptides, non-peptides, peptidomimetica, immunoglobulins, such as antibodies or functional fragments thereof, or immunoconjugates (fusion proteins).

Preferred integrin inhibitors of the invention are directed to receptor of α_v integrins (e.g. $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_6$ and sub-classes). Preferred integrin inhibitors are α_v antagonists, and in particular $\alpha_v\beta_3$ antagonists. Preferred α_v antagonists according to the invention are RGD peptides, peptidomimetic (non-peptide) antagonists and anti-integrin receptor antibodies such as antibodies blocking α_v receptors.

Exemplary, non-immunological $\alpha_v\beta_3$ antagonists are described in the teachings of US 5,753,230 and US 5,766,591. Preferred antagonists are linear and cyclic RGD-containing peptides. Cyclic peptides are, as a rule, more stable and elicit an enhanced serum half-life. The most preferred integrin antagonist of the invention is, however, cyclo-(Arg-Gly-Asp-DPhe-NMeVal) (EMD 121974, Cilengitide[®], Merck KgaA, Germany; EP 0770 622) which is efficacious in blocking the integrin receptors $\alpha_v\beta_3$, $\alpha_v\beta_1$, $\alpha_v\beta_6$, $\alpha_v\beta_8$, $\alpha_{IIb}\beta_3$.

Suitable peptidic as well as peptido-mimetic (non-peptide) antagonists of the $\alpha_v\beta_3$ / $\alpha_v\beta_5$ / $\alpha_v\beta_6$ integrin receptor have been described both in the scientific and patent literature. For example, reference is made to Hoekstra and Poulter, 1998, Curr. Med. Chem. 5, 195; WO 95/32710; WO 95/37655; WO 97/01540; WO 97/37655; WO 97/45137; WO 97/41844; WO 98/08840; WO 98/18460; WO 98/18461; WO 98/25892; WO 98/31359; WO 98/30542; WO 99/15506; WO 99/15507; WO 99/31061; WO 00/06169; EP 0853 084; EP 0854 140; EP 0854 145; US 5,780,426; and US 6,048,861. Patents that disclose benzazepine, as well as related benzodiazepine and benzocycloheptene $\alpha_v\beta_3$ integrin

receptor antagonists, which are also suitable for the use in this invention, include WO 96/00574, WO 96/00730, WO 96/06087, WO 96/26190, WO 97/24119, WO 97/24122, WO 97/24124, WO 98/15278, WO 99/05107, WO 99/06049, WO 99/15170, WO 99/15178, WO 97/34865, WO 97/01540, WO 98/30542, WO 5 99/11626, and WO 99/15508. Other integrin receptor antagonists featuring backbone conformational ring constraints have been described in WO 98/08840; WO 99/30709; WO 99/30713; WO 99/31099; WO 00/09503; US 5,919,792; US 5,925,655; US 5,981,546; and US 6,017,926. In US 6,048,861 and WO 00/72801 a series of nonanoic acid derivatives which are potent $\alpha_v\beta_3$ integrin receptor 10 antagonists were disclosed. Other chemical small molecule integrin antagonists (mostly vitronectin antagonists) are described in WO 00/38665. Other $\alpha_v\beta_3$ receptor antagonists have been shown to be effective in inhibiting angiogenesis. For example, synthetic receptor antagonists such as (S)-10,11-Dihydro-3-[3-(pyridin-2-ylamino)-1-propyloxy]-5H-dibenzo[a,d]cycloheptene-10- 15 acetic acid (known as SB-265123) have been tested in a variety of mammalian model systems. (Keenan et al., 1998, Bioorg. Med. Chem. Lett. 8(22), 3171; Ward et al., 1999, Drug Metab. Dispos. 27(11),1232). Assays for the identification of integrin antagonists suitable for use as an antagonist are described, e.g. by Smith et al., 1990, J. Biol. Chem. 265, 12267, and in the referenced patent 20 literature.

Anti-integrin receptor antibodies are also well known. Suitable anti-integrin (e.g. $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_6$) monoclonal antibodies can be modified to encompass antigen binding fragments thereof, including F(ab)₂, Fab, and engineered Fv or single-chain antibody. One suitable and preferably used monoclonal antibody directed 25 against integrin receptor $\alpha_v\beta_3$ is identified as LM609 (Brooks et al., 1994, Cell 79, 1157; ATCC HB 9537). A potent specific anti- $\alpha_v\beta_5$ antibody, P1F6, is disclosed in WO 97/45447, which is also preferred according to this invention. A further suitable $\alpha_v\beta_6$ selective antibody is MAb 14D9.F8 (WO 99/37683, DSM ACC2331, Merck KGaA, Germany) as well as MAb 17.E6 (EP 0719 859, DSM ACC2160, 30 Merck KGaA) which is selectively directed to the α_v - chain of integrin receptors. Another suitable anti-integrin antibody is the commercialized Vitroxin ®.

A "**angiogenic growth factor or growth factor receptor**" is a factor or receptor which promotes by its activation the growth and development of blood vessels. Typically, Vascular Endothelial Growth Factor (VEGF) and its receptor belong to this group.

5

The term "**antibody**" or "**immunoglobulin**" herein is used in the broadest sense and specifically covers intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g. bispecific antibodies) formed from at least two intact antibodies, and antibody fragments, so long as they exhibit the desired biological activity. The term generally includes heteroantibodies which are composed of two or more antibodies or fragments thereof of different binding specificity which are linked together.

10

Depending on the amino acid sequence of their constant regions, intact antibodies can be assigned to different "**antibody (immunoglobulin) classes**".

15

There are five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into "subclasses" (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain constant domains that correspond to the different classes of antibodies are called α , δ , ϵ , γ and μ respectively. Preferred major class for antibodies according to the invention is

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IgG, in more detail IgG1 and IgG2.

Antibodies are usually glycoproteins having a molecular weight of about 150,000, composed of two identical light (L) chains and two identical heavy (H) chains.

Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different

25

immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (VH) followed by a number of constant domains. Each light chain has a variable domain at one end (VL) and a constant domain at its other end. The constant domain of the light chain is aligned with the first constant domain of the heavy

30

chain, and the light-chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains. The "light chains" of antibodies from any vertebrate species can be assigned to one of two clearly

distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

The term "**monoclonal antibody**" as used herein refers to an antibody obtained
5 from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations which include
10 different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. Methods for making monoclonal antibodies include the hybridoma method described by
15 Kohler and Milstein (1975, *Nature* 256, 495) and in "Monoclonal Antibody Technology, The Production and Characterization of Rodent and Human Hybridomas" (1985, Burdon et al., Eds, Laboratory Techniques in Biochemistry and Molecular Biology, Volume 13, Elsevier Science Publishers, Amsterdam), or may be made by well known recombinant DNA methods (see, e.g., US
20 4,816,567). Monoclonal antibodies may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.*, *Nature*, 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.*, 222:58, 1-597(1991), for example.

The term "**chimeric antibody**" means antibodies in which a portion of the heavy
25 and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments
30 of such antibodies, so long as they exhibit the desired biological activity (e.g.: US 4,816,567; Morrison et al., *Proc. Nat. Acad. Sci. USA*, 81:6851-6855 (1984)). Methods for making chimeric and humanized antibodies are also known in the art. For example, methods for making chimeric antibodies include those

described in patents by Boss (Celltech) and by Cabilly (Genentech) (US 4,816,397; US 4,816,567).

- "Humanized antibodies"** are forms of non-human (e.g., rodent) chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region (CDRs) of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity and capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. Methods for making humanized antibodies are described, for example, by Winter (US 5,225,539) and Boss (Celltech, US 4,816,397).
- The term **"variable"** or **"FR"** refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called hypervariable regions both in the light chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FRs). The variable domains of native heavy and light chains each comprise four FRs (FR1 – FR4), largely adopting a β -sheet configuration, connected by three

hypervariable regions, which form loops connecting, and in some cases forming part of, the β -sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC).

10

The term "**hypervariable region**" or "**CDR**" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region generally comprises amino acid residues from a "complementarity determining region" or "CDR" (e.g. residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; and/or those residues from a "hypervariable loop" (e.g. residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)).

20

"**Framework Region**" or "**FR**" residues are those variable domain residues other than the hypervariable region residues as herein defined.

"**Antibody fragments**" comprise a portion of an intact antibody, preferably comprising the antigen-binding or variable region thereof. Examples of antibody fragments include Fab, Fab', F(ab')₂, Fv and Fc fragments, diabodies, linear antibodies, single-chain antibody molecules; and multispecific antibodies formed from antibody fragment(s). An "intact" antibody is one which comprises an antigen-binding variable region as well as a light chain constant domain (CL) and heavy chain constant domains, CH1, CH2 and CH3. Preferably, the intact antibody has one or more effector functions.

30

Papain digestion of antibodies produces two identical antigen-binding fragments, called "**Fab**" fragments, each comprising a single antigen-binding site and a CL

and a CH1 region, and a residual "**Fc**" fragment, whose name reflects its ability to crystallize readily.

The "**Fc**" region of the antibodies comprises, as a rule, a CH2, CH3 and the hinge region of an IgG1 or IgG2 antibody major class. The hinge region is a group of
5 about 15 amino acid residues which combine the CH1 region with the CH2-CH3 region.

Pepsin treatment yields an "**F(ab')₂**" fragment that has two antigen-binding sites and is still capable of cross-linking antigen. "**Fv**" is the minimum antibody fragment which contains a complete antigen-recognition and antigen-binding site.

10 This region consists of a dimer of one heavy chain and one light chain variable domain in tight, non-covalent association. It is in this configuration that the three hypervariable regions (CDRs) of each variable domain interact to define an antigen-binding site on the surface of the VH - VL dimer. Collectively, the six hypervariable regions confer antigen-binding specificity to the antibody. However,
15 even a single variable domain (or half of an Fv comprising only three hypervariable regions specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site. The **Fab** fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. "**Fab'**" fragments differ from Fab
20 fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. F(ab')₂ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known (see e.g. Hermanson, Bioconjugate
25 Techniques, Academic Press, 1996; . US 4,342,566).

"**Single-chain Fv**" or "**scFv**" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a Single polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the scFv to form the desired structure for
30 antigen binding. Single-chain FV antibodies are known, for example, from Plückthun (*The Pharmacology of Monoclonal Antibodies*, Vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994)), WO93/16185;

US 5,571,894; US 5,587,458; Huston et al. (1988, Proc.Natl. Acad. Sci. 85, 5879) or Skerra and Plueckthun (1988, Science 240, 1038).

The term "**diabodies**" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a variable heavy domain (V_H) connected to a
5 variable light domain (V_L) in the same polypeptide chain (V_H - V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161.

10

"**Bispecific antibodies**" are single, divalent antibodies (or immunotherapeutically effective fragments thereof) which have two differently specific antigen binding sites. For example the first antigen binding site is directed to an angiogenesis receptor (e.g. integrin or VEGF receptor), whereas the second
15 antigen binding site is directed to an ErbB receptor (e.g. EGFR or HER2).

Bispecific antibodies can be produced by chemical techniques (see e.g., Kranz et al. (1981) Proc. Natl. Acad. Sci. USA 78, 5807), by "polydoma" techniques (See US 4,474,893) or by recombinant DNA techniques, which all are known per se. Further methods are described in WO 91/00360, WO 92/05793 and WO
20 96/04305. Bispecific antibodies can also be prepared from single chain antibodies (see e.g., Huston et al. (1988) Proc. Natl. Acad. Sci. 85, 5879; Skerra and Plueckthun (1988) Science 240, 1038). These are analogues of antibody variable regions produced as a single polypeptide chain. To form the bispecific binding agent, the single chain antibodies may be coupled together chemically or by
25 genetic engineering methods known in the art. It is also possible to produce bispecific antibodies according to this invention by using leucine zipper sequences. The sequences employed are derived from the leucine zipper regions of the transcription factors Fos and Jun (Landschulz et al., 1988, Science 240,1759; for review, see Maniatis and Abel, 1989, Nature 341, 24). Leucine
30 zippers are specific amino acid sequences about 20-40 residues long with leucine typically occurring at every seventh residue. Such zipper sequences form amphipathic α -helices, with the leucine residues lined up on the hydrophobic side for dimer formation. Peptides corresponding to the leucine zippers of the Fos and

Jun proteins form heterodimers preferentially (O'Shea et al., 1989, Science 245, 646). Zipper containing bispecific antibodies and methods for making them are also disclosed in WO 92/10209 and WO 93/11162. A bispecific antibody according the invention may be an antibody, directed to VEGF receptor and
5 α V β 3 receptor as discussed above with respect to the antibodies having single specificity.

The term "**immunoconjugate**" refers to an antibody or immunoglobulin, respectively, or a immunologically effective fragment thereof, which is fused by
10 covalent linkage to a non-immunologically effective molecule. Preferably this fusion partner is a peptide or a protein, which may be glycosylated. Said non-antibody molecule can be linked to the C-terminal of the constant heavy chains of the antibody or to the N-terminals of the variable light and/or heavy chains. The fusion partners can be linked via a linker molecule, which is, as a rule, a 3 – 15
15 amino acid residues containing peptide. Immunoconjugates according to the invention comprise preferably fusion proteins consisting of an immunoglobulin or immunotherapeutically effective fragment thereof, directed to an angiogenic receptor, preferably an integrin or VEGF receptor and TNF α or a fusion protein consisting essentially of TNF α and IFN γ or another suitable cytokine, which is
20 linked with its N-terminal to the C-terminal of said immunoglobulin, preferably the Fc portion thereof.

The term "**fusion protein**" refers to a natural or synthetic molecule consisting of one ore more non-immunotherapeutically effective (non-antibody) proteins or
25 peptides having different specificity which are fused together optionally by a linker molecule. Fusion protein according to the invention may be molecules consisting of, for example, cyclo-(Arg-Gly-Asp-DPhe-NMeVal) fused to TNF α and / or IFN γ .

"**Heteroantibodies**" are two or more antibodies or antibody-binding fragments
30 which are linked together, each of them having a different binding specificity. Heteroantibodies can be prepared by conjugating together two or more antibodies or antibody fragments. Preferred heteroantibodies are comprised of cross-linked Fab/Fab' fragments. A variety of coupling or crosslinking agents can

be used to conjugate the antibodies. Examples are protein A, carbodiimide, N-succinimidyl-S-acetyl-thioacetate (SATA) and N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP) (see e.g., Karpovsky et al. (1984) J. EXP. Med. 160, 1686; Liu et al. (1985) Proc. Natl. Acad. Sci. USA 82, 8648). Other methods
5 include those described by Paulus, Behring Inst. Mitt., No. 78, 118 (1985); Brennan et al. (1985) Science 30 m:81 or Glennie et al. (1987) J. Immunol. 139, 2367. Another method uses o-phenylenedimaleimide (oPDM) for coupling three Fab' fragments (WO 91/03493). Multispecific antibodies are in context of this invention also suitable and can be prepared, for example according to the
10 teaching of WO 94/13804 and WO 98/50431.

Antibody "**effector functions**" refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody. Examples of antibody effector functions include
15 complement dependent cytotoxicity, Fc receptor binding, antibody-dependent cell-mediated cytotoxicity (ADCC), phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor), etc.

The term "**ADCC**" (antibody-dependent cell-mediated cytotoxicity) refers to a cell-
20 mediated reaction in which nonspecific cytotoxic cells that express Fc receptors (FcR) (e.g. natural killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and subsequently cause lysis of the target cell. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. To assess ADCC activity of a
25 molecule of interest, an *in vitro* ADCC assay, such as that described in the prior art (US 5,500,362; US 5,821,337) may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and natural killer (NK) cells.

30 "**Human effector cells**" are leukocytes which express one or more FcRs and perform effector functions. Preferably, the cells express at least FcγRIII and perform ADCC effector function. Examples of human leukocytes which mediate

ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, cytotoxic T cells and neutrophils.

The terms "**Fc receptor**" or "FcR" are used to describe a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcRs are reviewed, for example, in Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991).

The term "**cytokine**" is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor (VEGF); integrin; thrombopoietin (TPO); nerve growth factors such as NGFβ; platelet-growth factor; transforming growth factors (TGFs) such as TGFα and TGFβ; erythropoietin (EPO); interferons such as IFNα, IFNβ, and IFNγ; colony stimulating factors such as M-CSF, GM-CSF and G-CSF; interleukins such as IL-1, IL-1a, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; and TNFα or TNFβ. Preferred cytokines according to the invention are interferons and TNFα.

The term "**cytotoxic agent**" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes, chemotherapeutic agents, and toxins such as enzymatically active toxins of bacterial, fungal, plant or animal origin, or

fragments thereof. The term may include also members of the cytokine family, preferably IFN γ as well as anti-neoplastic agents having also cytotoxic activity.

The term "**chemotherapeutic agent**" or "**anti-neoplastic agent**" includes according to this invention chemical agents that exert anti-neoplastic effects, i.e., prevent the development, maturation, or spread of neoplastic cells, directly on the tumor cell, e.g., by cytostatic or cytotoxic effects, and not indirectly through mechanisms such as biological response modification. Suitable chemotherapeutic agents according to the invention are preferably natural or synthetic chemical compounds, but biological molecules, such as proteins, polypeptides etc. are not expressively excluded. There are large numbers of anti-neoplastic agents available in commercial use, in clinical evaluation and in pre-clinical development, which could be included in the present invention for treatment of tumors / neoplasia by combination therapy with TNF α and the anti-angiogenic agents as cited above, optionally with other agents such as EGF receptor antagonists. It should be pointed out that the chemotherapeutic agents can be administered optionally together with above-said drug combination. Examples of chemotherapeutic agents include alkylating agents, for example, nitrogen mustards, ethyleneimine compounds, alkyl sulphonates and other compounds with an alkylating action such as nitrosoureas, cisplatin and dacarbazine; antimetabolites, for example, folic acid, purine or pyrimidine antagonists; mitotic inhibitors, for example, vinca alkaloids and derivatives of podophyllotoxin; cytotoxic antibiotics and camptothecin derivatives. Preferred chemotherapeutic agents or chemotherapy include amifostine (ethyol), cisplatin, dacarbazine (DTIC), dactinomycin, mechlorethamine (nitrogen mustard), streptozocin, cyclophosphamide, carmustine (BCNU), lomustine (CCNU), doxorubicin (adriamycin), doxorubicin lipo (doxil), gemcitabine (gemzar), daunorubicin, daunorubicin lipo (daunoxome), procarbazine, mitomycin, cytarabine, etoposide, methotrexate, 5-fluorouracil (5-FU), vinblastine, vincristine, bleomycin, paclitaxel (taxol), docetaxel (taxotere), aldesleukin, asparaginase, busulfan, carboplatin, cladribine, camptothecin, CPT-11, 10-hydroxy-7-ethyl-camptothecin (SN38), dacarbazine, floxuridine, fludarabine, hydroxyurea, ifosfamide, idarubicin, mesna, interferon alpha,

interferon beta, irinotecan, mitoxantrone, topotecan, leuprolide, megestrol, melphalan, mercaptopurine, plicamycin, mitotane, pegaspargase, pentostatin, pipobroman, plicamycin, streptozocin, tamoxifen, teniposide, testolactone, thioguanine, thiotepa, uracil mustard, vinorelbine, chlorambucil and combinations thereof.

Most preferred chemotherapeutic agents according to the invention are cisplatin, gemcitabine, doxorubicin, paclitaxel (taxol) and bleomycin.

The terms "**cancer**" and "**tumor**" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. By means of the pharmaceutical compositions according to the present invention tumors can be treated such as tumors of the breast, heart, lung, small intestine, colon, spleen, kidney, bladder, head and neck, ovary, prostate, brain, pancreas, skin, bone, bone marrow, blood, thymus, uterus, testicles, cervix, and liver. More specifically the tumor is selected from the group consisting of adenoma, angiosarcoma, astrocytoma, epithelial carcinoma, germinoma, glioblastoma, glioma, hamartoma, hemangioendothelioma, hemangiosarcoma, hematoma, hepatoblastoma, leukemia, lymphoma, medulloblastoma, melanoma, neuroblastoma, osteosarcoma, retinoblastoma, rhabdomyosarcoma, sarcoma and teratoma. In detail, the tumor is selected from the group consisting of acral lentiginous melanoma, actinic keratoses, adenocarcinoma, adenoid cystic carcinoma, adenomas, adenosarcoma, adenosquamous carcinoma, astrocytic tumors, Bartholin gland carcinoma, basal cell carcinoma, bronchial gland carcinomas, capillary, carcinoids, carcinoma, carcinosarcoma, cavernous, cholangiocarcinoma, chondrosarcoma, choroid plexus papilloma/carcinoma, clear cell carcinoma, cystadenoma, endodermal sinus tumor, endometrial hyperplasia, endometrial stromal sarcoma, endometrioid adenocarcinoma, ependymal, epithelioid, Ewing's sarcoma, fibrolamellar, focal nodular hyperplasia, gastrinoma, germ cell tumors, glioblastoma, glucagonoma, hemangiblastomas, hemangioendothelioma, hemangiomas, hepatic adenoma, hepatic adenomatosis, hepatocellular carcinoma, insulinoma, intraepithelial neoplasia, interepithelial squamous cell neoplasia, invasive squamous cell carcinoma, large cell carcinoma, leiomyosarcoma, lentigo maligna melanomas, malignant melanoma,

malignant mesothelial tumors, medulloblastoma, medulloepithelioma, melanoma, meningeal, mesothelial, metastatic carcinoma, mucoepidermoid carcinoma, neuroblastoma, neuroepithelial adenocarcinoma nodular melanoma, oat cell carcinoma, oligodendroglial, osteosarcoma, pancreatic polypeptide, papillary
5 serous adeno-carcinoma, pineal cell, pituitary tumors, plasmacytoma, pseudo-sarcoma, pulmonary blastoma, renal cell carcinoma, retinoblastoma, rhabdomyo-sarcoma, sarcoma, serous carcinoma, small cell carcinoma, soft tissue carcinomas, somatostatin-secreting tumor, squamous carcinoma, squamous cell carcinoma, submesothelial, superficial spreading melanoma, undifferentiated
10 carcinoma, uveal melanoma, verrucous carcinoma, vipoma, well differentiated carcinoma, and Wilm's tumor.

An "**ErbB receptor**" is a receptor protein tyrosine kinase which belongs to the ErbB receptor family and includes EGFR(ErbB1), ErbB2, ErbB3 and ErbB4
15 receptors and other members of this family to be identified in the future. The ErbB receptor will generally comprise an extracellular domain, which may bind an ErbB ligand; a lipophilic transmembrane domain; a conserved intracellular tyrosine kinase domain; and a carboxyl-terminal signaling domain harboring several tyrosine residues which can be phosphorylated. The ErbB receptor may be a
20 "native sequence" ErbB receptor or an "amino acid sequence variant" thereof. Preferably the ErbB receptor is native sequence human ErbB receptor. ErbB1 refers to the gene encoding the EGFR protein product. Mostly preferred is the EGF receptor (HER1). The expressions "ErbB1" and "HER1" are used interchangeably herein and refer to human HER1 protein. The expressions
25 "ErbB2" and "HER2" are used interchangeably herein and refer to human HER2 protein. ErbB1 receptors (EGFR) are preferred according to this invention

"**ErbB ligand**" is a polypeptide which binds to and/or activates an ErbB receptor. ErbB ligands which bind EGFR include EGF, TGF- α , amphiregulin, betacellulin,
30 HB-EGF and epiregulin.

The term "**ErbB receptor antagonist / inhibitor**" refers to a natural or synthetic molecule which binds and blocks or inhibits the ErbB receptor. Thus, by blocking

the receptor the antagonist prevents binding of the ErbB ligand (agonist) and activation of the agonist/ligand receptor complex. ErbB antagonists may be directed to HER1 (EGFR) or HER2. Preferred antagonists of the invention are directed to the EGF receptor (EGFR, HER1). The ErbB receptor antagonist may
5 be an antibody or an immunotherapeutically effective fragment thereof or non-immunobiological molecules, such as a peptide, polypeptide protein. Chemical molecules are also included, however, anti-EGFR antibodies and anti-HER2 antibodies are the preferred antagonists according to the invention.

Preferred antibodies of the invention are anti-Her1 and anti-Her2 antibodies,
10 more preferably anti-Her1 antibodies. Preferred anti-Her1 antibodies are MAb 425, preferably humanized MAb 425 (hMAb 425, US 5,558,864; EP 0531 472) and chimeric MAb 225 (cMAb 225, US 4,943,533 and EP 0359 282). Most preferred is monoclonal antibody h425, which has shown in mono-drug therapy high efficacy combined with reduced adverse and side effects. Most preferred

15 anti-HER2 antibody is HERCEPTIN[®] commercialized by Genentech/Roche.

Efficacious EGF receptor antagonists according to the invention may be also natural or synthetic chemical compounds. Some examples of preferred molecules of this category include organic compounds, organometallic compounds, salts of organic and organometallic compounds.

20 Examples for HER2 receptor antagonists are: styryl substituted heteroaryl compounds (US 5,656,655); bis mono and/or bicyclic aryl heteroaryl, carbocyclic, and heterocarbocyclic compounds (US 5,646,153); tricyclic pyrimidine compounds (US 5,679,683); quinazoline derivatives having receptor tyrosine kinase inhibitory activity (US 5,616,582); heteroarylethenediyl or heteroaryl-
25 ethenediylaryl compounds (US 5,196,446); a compound designated as 6-(2,6-dichlorophenyl)-2-(4-(2-diethyl-aminoethoxy) phenylamino)-8-methyl-8H-pyrido(2,3)-5-pyrimidin-7-one (Panek, et al., 1997, J. Pharmacol. Exp. Therap. 283,1433) inhibiting EGFR, PDGFR, and FGFR families of receptors.

30 The term "tyrosine kinase antagonist/inhibitor" refers to natural or synthetic agents that are enabled to inhibit or block tyrosine kinases, receptor tyrosine kinases included. With exception of the anti-ErbB receptor antibodies mentioned above and below, more preferable tyrosine kinase antagonist agents are

chemical compounds which have shown efficacy in mono- drug therapy for breast and prostate cancer. Suitable indolocarbazole-type tyrosine kinase inhibitors can be obtained using information found in documents such as US patents 5,516,771; 5,654,427; 5,461,146; 5,650,407. US patents 5,475,110; 5,591,855; 5,594,009 and WO 96/11933 disclose pyrrolocarbazole-type tyrosine kinase inhibitors and prostate cancer. Preferably, the dosage of the chemical tyrosine kinase inhibitors as defined above is from 1 pg/kg to 1 g/kg of body weight per day. More preferably, the dosage of tyrosine kinase inhibitors is from 0.01 mg/kg to 100 mg/kg of body weight per day.

10

As used herein, the term "**anti-hormonal agent**" includes natural or synthetic organic or peptidic compounds that act to regulate or inhibit hormone action on tumors. In more detail an "anti-hormonal agent" (1) inhibits the production of serum androgens, (2) blocks binding of serum androgens to androgen receptors, or (3) inhibits the conversion of testosterone to DHT, or a combination of two or more such compounds.

An anti-hormonal agent according to the invention includes in general steroid receptor antagonists and in more detail anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY 117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above. The term includes also agonists and / or antagonists of glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH) and LHRH (luteinizing hormone-releasing hormone). A LHRH agonist useful in this invention is goserelin acetate, commercially available as ZOLADEX® (Zeneca). The chemical structure of goserelin acetate is pyro-Glu-His-Trp-Ser-Tyr-D-Ser(But)-Leu-Arg-Pro-Azgly-NH, acetate. An example of an LHRH antagonist useful in this invention is ANTIDEX® (Ares-Serono), whose chemical name is D-alaninamide N-acetyl-3-(2-naphthalenyl)-D-alanyl-4-chloro-D-phenylalanyl-3-(3-pyridinyl)-D-alanyl-L-seryl-NG-(3-pyridinylcarbonyl)-L-lysyl-N6-(3-pyridinylcarbonyl)-D-lysyl-L-leucyl-N6-(1-methylethyl)-L-lysyl-L-prolyl. Another

- example of a useful LHRH antagonist is GANIRELIX® (Roche/Akzo Nobel), whose chemical name is N-Ac-D-Nal,D-pCl-Phe,D-Pal,D-hArg(Et)₂,hArg(Et)₂,D-Ala. Examples of steroidal anti-androgens are cyproterone acetate (CPA) and megestrol acetate, commercially available as MEGACE® (Bristol-Myers
- 5 Oncology). Steroidal anti-androgens may block prostatic androgen receptors. They may also inhibit the release of LH. CPA is preferably administered to human patients at dosages of 100 mg/day to 250 mg/day. Nonsteroidal anti-androgens block androgen receptors. They may also cause an increase in serum LH levels and Serum testosterone levels. A preferred nonsteroidal anti-androgen is
- 10 flutamide (2-methyl-N-[4- 20 nitro-3-(trifluoromethyl)phenyl] propanamide), commercially available as EULEXIN® (Schering Corp.). Flutamide exerts is anti-androgenic action by inhibiting androgen uptake, by inhibiting nuclear binding of androgen in target tissues, or both. Another non-steroidal anti-androgen is nilutamide, whose chemical name is 5,5-dimethyl-3-[4-nitro-3- (trifluoromethyl -4'-
- 15 nitrophenyl)-4,4- dimethyl-imidazolidine-dione.
- In some embodiments of the invention, the anti-hormonal agent is a combination of an LHRH agonist such as leuprolide acetate, and an antiandrogen such as flutamide or nilutamide. For example, leuprolide acetate can be administered by subcutaneous, intramuscular or intravenous injection, and concurrently the
- 20 flutamide can be administered orally.
- Anti-hormonal agents according to the invention include, as pointed out above, antagonists of the steroid/thyroid hormone receptors, including antagonists for other non-permissive receptors, such as antagonists for RAR, TR, VDR, and the like. As readily recognized by those of skill in the art, a variety of retinoic acid
- 25 receptor (RAR) antagonists, both synthetic and naturally occurring, can be used in accordance with the present invention. Exemplary RAR antagonists include dicarba-closo-dodecaboranes (Iijima et al., Chem Pharm Bull (Tokyo) (1999) 47(3):398-404), hydroanthracenyl, benzochromenyl and benzothiochromenyl retinoids (Vuligonda et al., Bioorg Med Chem Lett (1999) 9(5):743-8),
- 30 diarylacetylenes, benzoic acid derivatives (see, e.g., Kagechika, H. (1994) Yakugaku Zasshi 114(11):847-862; Eckhardt et al. (1994) Toxicol Lett 70(3):299-308; Yoshimura et al. (1995) J Med Chem 38(16):3163-3 173; 30 Chen et al. (1995) EMBO 14(6):1187-1197; Teng et al. (1997) J Med Chem 40(16):2445-

2451); naphthalenyl analogs (see, e.g., Johnson et al. (1995) *J Med Chem* 38(24):4764-4767; Agarwal et al. *J Biol Chem* 271(21): 12209-12212; Umemiya et al. (1996) *Yakugaku Zasshi* 116(12):928-941); aryl-substituted and aryl and (3-oxo-1-propenyl)-substituted benzopyran, benzothiopyran, 1,2-dihydroquinoline, and 5,6-dihydronaphthalene derivatives (Klein et al. U.S. Pat. Nos. 5877,207 and 5,776,699), adamantyl-substituted biaromatic compounds (Bernardon and Charpentier, U.S. Pat. No. 5,877,342), 1-phenyl-adamancane derivatives (Bernardon and Bernardon EP 776885), polyaromatic heterocyclic compounds (Charpentier et al. U.S. Pat. No. 5,849,798), dihydronaphthalene derivatives (Beard et al., U.S. Pat. No. 5,808,124 and Johnson et al. US 5,773,594), 4-phenyl (benzopyranoyl or naphthoyl) amidobenzoic acid derivatives (WO 98/US/13065), diazepinylbenzoic acid derivatives (Umemiya et al., *J Med Chem* (1997) 40(26):4222-34), tetrahydronaphthalene derivatives (US 5,763,635, 5,741,896 and 5,723,666), aryl-and heteroarylcyclohexenyl substituted alkenes (US 5,760,276), dibenzofuran compounds, including aromatic dibenzofuran compounds (US 5,702,710, US 5,747,530), N-aryl substituted tetrahydroquinolines (US 5,739,338), benzo[1,2-g]-Chrom-3-ene and benzo[1,2-g]-thiochrom-3-ene derivatives (US 5,728,846), and the like. Examples of specific RAR antagonists contemplated for use herein include LE135 (Umemiya et al. (1996) *Yakugaku Zasshi* 116(12):928-941), LE511, LE540, LE550 (Li et al., *J Biol Chem* (1999) 274(22): 15360-6; Umemiya et al. (1996) *Yakugaku Zasshi* 116(12):928-941), Ro41-5253 (Keidel et al. (1994) *Mol Cell Biol* 14(1):287-298), SR11330, SR11334, SR11335 (Lee et al. (1996) *J. Biol Chem* 271(20):11897-11903), BMS453, BMS411 (Chen et al. (1995) *EMBO* 14(6):1187- 1197), CD2366 and CD2665 (Meister et al., *Anticancer Res.* (1998) 18(3A): 1777-1786), ER27191 (Uemo et al., *Leuk. Res.* (1998) 22(6):5 17-525), AGN 193 109 (Johnson et al., *Bioorg Med Chem Lett* (1999) 9(4):573-6), 4-[4,5,7,8,9,10-hexahydro-7,7,10,10-tetramethyl-1-(3-pyridylmethyl)anthra[1,2-b]pyrrol-3-yl]benzoic acid, 4-[4,5,7,8,9,10-hexahydro-7,7,10,10-tetramethyl-1-(3-pyridylmethyl)-5-thiaanthra[1,2-b]pyrrol-3-yl]benzoic acid, 4-[4,5,7,8,9,10-hexahydro-7,7,10,10-tetramethyl-1-(3-pyridylmethyl)anthra[2,1-d]pyrazol-3-yl]benzoic acid (Yoshimura et al. (1995) *J Med Chem* 38(16):3163-3173), AGN193109 (Agarwal et al. *J Biol Chem* 271(21):12209-12212), and the like.

By "**steroid receptor**" or "**nuclear steroid receptor**" is meant a protein that is a ligand-activated transcription factor, and belongs to the steroid receptor subfamily of nuclear receptors. Included in the definition of steroid receptors are proteins which -structurally resemble and have the biological activity of a steroid hormone-
5 activated transcription factor. Steroid receptors contain all or part of a DNA binding domain and a hormone (or ligand) binding domain, and include orphan receptors for unknown ligands whose structure resembles that of steroid receptors.

10 By "**steroid receptor ligand**" is meant a natural or synthetic compound which binds the nuclear steroid receptor to form a receptor-ligand complex. The term ligand includes agonists, antagonists, and compounds with partial agonist/antagonist action.

15 By "**steroid receptor agonist**" is meant a compound which binds the nuclear steroid receptor to form a receptor-agonist complex. The receptor-agonist complex binds specific regions of DNA termed hormone response elements. Agonists include steroid or steroid-like hormone, retinoids, thyroid hormones, pharmaceutically active compounds, and the like. Individual agonists may have
20 the ability to bind to multiple receptors. Natural steroid hormone agonists include estradiol, progesterone, androgens, glucocorticoids, and mineralocorticoids. As pointed out above some steroid receptor agonists may show some efficiency as antagonists as a function of dosage. Therefore, such an "agonist" may be effective as an anti-hormonal agent as defined according to this invention.

25

By "**steroid receptor antagonist**" is meant a compound that has a biological effect opposite to that of an agonist. An antagonist binds the nuclear steroid receptor and blocks the action of a steroid receptor agonist by competing with the steroid agonist for receptor. An antagonist is defined by its ability to
30 block the actions of an agonist. Steroid receptor antagonists include "pure" antagonists, as well as compounds with partial agonist/antagonist action. A pure antagonist effectively competes with an agonist for receptor binding, without itself having agonist actions. A partial antagonist may be less effective at

competing with an agonist for receptor binding, or may be equally effective at binding the receptor but have only 5-10% of the agonist action than that of the agonist being competed with. Thus, an antagonist may have an agonist effect less effective than that of the competing agonist.

5

"Radiotherapy": According to the invention the tumors can additionally be treated with radiation or radiopharmaceuticals. The source of radiation can be either external or internal to the patient being treated. When the source is external to the patient, the therapy is known as external beam radiation therapy

10 (EBRT). When the source of radiation is internal to the patient, the treatment is called brachytherapy (BT). Some typical radioactive atoms that have been used include radium, cesium-137, and iridium-192, americium-241 and gold-198, Cobalt-57; Copper-67; Technetium-99; Iodide-123; Iodide-131; and Indium-111. It is also possible to label the agents according to the invention with radioactive
15 isotopes.

Today radiation therapy is the standard treatment to control unresectable or inoperable tumors and / or tumor metastases. Improved results have been seen when radiation therapy has been combined with chemotherapy. Radiation therapy is based on the principle that high-dose radiation delivered to a target
20 area will result in the death of reproductive cells in both tumor and normal tissues. The radiation dosage regimen is generally defined in terms of radiation absorbed dose (rad) , time and fractionation, and must be carefully defined by the oncologist. The amount of radiation a patient receives will depend on various consideration but the two most important considerations are the location of the
25 tumor in relation to other critical structures or organs of the body, and the extent to which the tumor has spread. A preferred course of treatment for a patient undergoing radiation therapy will be a treatment schedule over a 5 to 6 week period, with a total dose of 50 to 60 Gy administered to the patient in a single daily fraction of 1.8 to 2.0 Gy, 5 days a week. A Gy is an abbreviation for
30 Gray and refers to 100 rad of dose. In the preferred embodiment, there is synergy when tumors in human patients are treated with the angiogenesis antagonist and TNF α /IFN γ and radiation. In other words, the inhibition of tumor growth by means of said compounds is enhanced when combined with radiation

and / or chemotherapeutic agents. Radiation therapy can be optionally used according to the invention. It is recommended and preferred in cases in which no sufficient amounts of the agents according to the invention can be administered to the patient.

5

"Pharmaceutical treatment": The method of the invention comprises a variety of modalities for practicing the invention in terms of the steps. For example, the agents according to the invention can be administered simultaneously, sequentially, or separately. Furthermore, the agents can be separately administered within a time interval of about 3 weeks between administrations, i.e., from substantially immediately after the first active agent is administered to up to about 3 weeks after the first agent is administered. The method can be practiced following a surgical procedure. Alternatively, the surgical procedure can be practiced during the interval between administration of the first active agent and the second active agent. Exemplary of this method is the combination of the present method with surgical tumor removal. Treatment according to the method will typically comprise administration of the therapeutic compositions in one or more cycles of administration. For example, where a simultaneous administration is practiced, a therapeutic composition comprising both agents is administered over a time period of from about 2 days to about 3 weeks in a single cycle. Thereafter, the treatment cycle can be repeated as needed according to the judgment of the practicing physician. Similarly, where a sequential application is contemplated, the administration time for each individual therapeutic will be adjusted to typically cover the same time period. The interval between cycles can vary from about zero to 2 months.

The agents of this invention can be administered parenterally by injection or by gradual infusion over time. Although the tissue to be treated can typically be accessed in the body by systemic administration and therefore most often treated by intravenous administration of therapeutic compositions, other tissues and delivery means are contemplated where there is a likelihood that the tissue targeted contains the target molecule. Thus, the agents of this invention can be administered intraocularly, intravenously, intraperitoneally, intramuscularly, subcutaneously, intracavity, transdermally, by orthotopic injection and infusion,

and can also be delivered by peristaltic means. The therapeutic compositions containing, for example, an integrin antagonist of this invention are conventionally administered intravenously, as by injection of a unit dose, for example.

Therapeutic compositions of the present invention contain a physiologically
5 tolerable carrier together with the relevant agent as described herein, dissolved or dispersed therein as an active ingredient. As used herein, the term
"pharmaceutically acceptable" refers to compositions, carriers, diluents and reagents which represent materials that are capable of administration to or upon a mammal without the production of undesirable physiological effects such as
10 nausea, dizziness, gastric upset and the like. The preparation of a pharmacological composition that contains active ingredients dissolved or dispersed therein is well understood in the art and need not be limited based on formulation. Typically, such compositions are prepared as injectables either as liquid solutions or suspensions, however, solid forms suitable for solution, or
15 suspensions, in liquid prior to use can also be prepared. The preparation can also be emulsified. The active ingredient can be mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient and in amounts suitable for use in the therapeutic methods described herein. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like
20 and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like which enhance the effectiveness of the active ingredient. The therapeutic composition of the present invention can include pharmaceutically acceptable salts of the components therein. Pharmaceutically
25 acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide) that are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, tartaric, mandelic and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium,
30 ammonium, calcium or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine and the like. Particularly preferred is the HCl salt when used in the preparation of cyclic polypeptide α v antagonists. Physiologically tolerable carriers are well known in

the art. Exemplary of liquid carriers are sterile aqueous solutions that contain no materials in addition to the active ingredients and water, or contain a buffer such as sodium phosphate at physiological pH value, physiological saline or both, such as phosphate-buffered saline. Still further, aqueous carriers can contain more
5 than one buffer salt, as well as salts such as sodium and potassium chlorides, dextrose, polyethylene glycol and other solutes. Liquid compositions can also contain liquid phases in addition to and to the exclusion of water. Exemplary of such additional liquid phases are glycerin, vegetable oils such as cottonseed oil, and water-oil emulsions.

10 Typically, a therapeutically effective amount of an immunotherapeutic agent, for example, in the form of an integrin receptor blocking antibody or antibody fragment or antibody conjugate or an anti-VEGF receptor blocking antibody, fragment or conjugate is an amount such that when administered in physiologically tolerable composition is sufficient to achieve a plasma
15 concentration of from about 0.01 microgram (μg) per milliliter (ml) to about 100 $\mu\text{g/ml}$, preferably from about 1 $\mu\text{g/ml}$ to about 5 $\mu\text{g/ml}$ and usually about 5 $\mu\text{g/ml}$. Stated differently, the dosage can vary from about 0.1 mg/kg to about 300 mg/kg, preferably from about 0.2 mg/kg to about 200 mg/kg, most preferably from about 0.5 mg/kg to about 20 mg/kg, in one or more dose administrations daily for one or
20 several days. Where the immunotherapeutic agent is in the form of a fragment of a monoclonal antibody or a conjugate, the amount can readily be adjusted based on the mass of the fragment / conjugate relative to the mass of the whole antibody. A preferred plasma concentration in molarity is from about 2 micromolar (μM) to about 5 millimolar (mM) and preferably, about 100 μM to 1 mM antibody
25 antagonist.

A therapeutically effective amount of an agent according of this invention which is a non-immunotherapeutic peptide or a protein polypeptide or other similarly-sized biological molecule, is typically an amount of polypeptide such that when administered in a physiologically tolerable composition is sufficient to achieve a
30 plasma concentration of from about 0.1 microgram (μg) per milliliter (ml) to about 200 $\mu\text{g/ml}$, preferably from about 1 $\mu\text{g/ml}$ to about 150 $\mu\text{g/ml}$. Based on a polypeptide having a mass of about 500 grams per mole, the preferred plasma

concentration in molarity is from about 2 micromolar (μM) to about 5 millimolar (mM) and preferably about 100 μM to 1 mM polypeptide antagonist.

The typical dosage of an active agent, which is a preferably a nuclear hormone
5 receptor antagonist or a (chemical) chemotherapeutic agent according to the invention (neither an immunotherapeutic agent nor a non-immunotherapeutic peptide/protein) is 10 mg to 1000 mg, preferably about 20 to 200 mg, and more preferably 50 to 100 mg per kilogram body weight per day.

10 The pharmaceutical compositions of this invention are preferably suitable for the treatment of breast and prostate cancer.

For breast cancer the following anti-hormonal agents and dosages are preferred:

	Tamoxifen:	1	0,0 mg – 40,0 mg p. o. / day
	Toremifen:		20,0 mg – 100,0 mg / day
15	Anastrozole:		0,5 mg – 5,0 mg p. o. / day
	Letrozole:		1,0 mg – 10,0 mg p. o. / day
	Formestane:		100,0 mg – 500,0 mg i. m. q 2 weeks
	Goserelin:		2,5 mg – 5,0 mg s. c. / 4 weeks
	Buserelin:		5,0 mg – 10,0 mg s. c. q 8 weeks
20			1,0 mg – 5,0 mg nasal spray / d
	Leuporelin:		5,0 mg – 15,0 mg s. c. q 3 months
	Megestrolacetate:		40,0 mg – 200,0 mg / d

For breast cancer the following anti-hormonal agents and dosages are preferred:

25	Flutamide:		250,0 mg – 1000,0 mg / d
	Bicalutamide:		10,0 mg – 200,0 mg / d
	Goserelin:		2,5 mg – 5,0 mg s. c. / 4 weeks
	Buserelin:		5,0 mg – 10,0 mg s. c. q 8 weeks
			1,0 mg – 5,0 mg nasal spray 7 d
30	Leuporelin:		5,0 mg – 15,0 mg s. c. q 3 months
	Cyproteronacetate:		25,0 mg – 200,0 mg / d p. o.
			150,0 mg – 500,0 mg i. m. q 2 weeks

Any analogs or further developments of the above-mentioned anti-hormone treatment principals will be used according to the proscribed doses.

The pharmaceutical compositions of the invention can comprise phrase
5 encompasses treatment of a subject with agents that reduce or avoid side effects associated with the combination therapy of the present invention ("adjunctive therapy"), including, but not limited to, those agents, for example, that reduce the toxic effect of anticancer drugs, e.g., bone resorption inhibitors, cardioprotective agents. Said adjunctive agents prevent or reduce the incidence of nausea and
10 vomiting associated with chemotherapy, radiotherapy or operation, or reduce the incidence of infection associated with the administration of myelosuppressive anticancer drugs. Adjunctive agents are well known in the art.

The immunotherapeutic agents according to the invention can additionally administered with adjuvants like BCG and immune system stimulators.

15 Furthermore, the compositions may include immunotherapeutic agents or chemotherapeutic agents which contain cytotoxic effective radio labeled isotopes, or other cytotoxic agents, such as a cytotoxic peptides (e.g. cytokines) or cytotoxic drugs and the like.

20 The term " **pharmaceutical kit**" for treating tumors or tumor metastases refers to a package and, as a rule, instructions for using the reagents in methods to treat tumors and tumor metastases. A reagent in a kit of this invention is typically formulated as a therapeutic composition as described herein, and therefore can be in any of a variety of forms suitable for distribution in a kit. Such
25 forms can include a liquid, powder, tablet, suspension and the like formulation for providing the antagonist and/or the fusion protein of the present invention. The reagents may be provided in separate containers suitable for administration separately according to the present methods, or alternatively may be provided combined in a composition in a single container in the package. The package
30 may contain an amount sufficient for one or more dosages of reagents according to the treatment methods described herein. A kit of this invention also contains "instruction for use" of the materials contained in the package.

Patent Claims

1. A pharmaceutical composition comprising in an therapeutically effective amount at least (i) one anti-EGFR antibody or an immunotherapeutically effective
5 fragment thereof and (ii) one anti-hormonal agent, optionally together with a pharmaceutically acceptable carrier, excipient or diluent.
2. A pharmaceutical composition according to claim 1, wherein said anti-EGFR antibody or said immunotherapeutically effective fragment thereof is murine,
10 chimeric or humanized Mab 425 (h425) or chimeric Mab 225 (c225) inhibitor/antagonist.
3. A pharmaceutical composition according to claim 1, wherein said anti-hormonal agent is an inhibitor of the nuclear hormone receptor family.
15
4. A pharmaceutical composition according to claim 3, wherein said anti-hormonal agent is an steroid receptor inhibitor/antagonist.
5. A pharmaceutical composition according to any of the claims 1 – 4 comprising
20 additionally an anti-angiogenic agent.
6. A pharmaceutical composition according to any of the claims 1 – 4, further comprising a cytotoxic and / or chemotherapeutic agent.
- 25 7. A pharmaceutical composition according to any of the claims 1 – 4, comprising additionally a further an anti-HER2 antibody or an immunotherapeutically active fragment thereof.
8. A pharmaceutical composition comprising an antibody having an anti-EGFR
30 activity and an anti-nuclear hormone receptor activity, optionally together with a pharmaceutically acceptable carrier, excipient or diluent..

9. A pharmaceutical composition of claim 8, wherein said antibody is a bispecific antibody.

10. A pharmaceutical kit comprising a package comprising at least

- 5 (i) one anti-EGFR antibody or an immunotherapeutically effective fragment thereof, and
(ii) one anti-hormonal agent, and optionally
(iii) a cytotoxic and/or chemotherapeutic agent.

10 11. A pharmaceutical kit of claim 10 comprising

- (i) monoclonal antibody h425, and
(ii) a steroid receptor antagonist

12. A pharmaceutical kit of claim 11 comprising

- 15 (i) monoclonal antibody h425, and
(ii) a LHRH antagonist

13. A pharmaceutical kit according to any of the claims 10 – 12, wherein said pharmaceutically active agents are provided in separate containers in said

20 package.

14. Use of a pharmaceutical composition as defined in any of the claims 1 – 9, or a pharmaceutical kit as defined in any of the claims 10 – 13, for the manufacture of a medicament or a composition of medicaments to treat tumors and tumor

25 metastases.

15. Use according to claim 14 for the treatment of breast cancer.

16. Use according to claim 14 for the treatment of prostate cancer.

30

17. Use according to claim 15 or 16 for the treatment of steroid-independent breast and prostate cancer.

18. A method for treating tumors or tumor metastases in an individual comprising administering to said individual simultaneously or sequentially a therapeutically effective amount of

- (i) an anti-EGFR antibody, and
- 5 (ii) an anti-hormonal agent.

19. A method of claim 18, wherein said anti-EGFR antibody is monoclonal antibody h425 or c225 and said anti-hormonal agent is a steroid receptor antagonist.

10

20. A method of claim 18 or 19 comprising administering additionally to said individual a therapeutically effective amount of a cytotoxic and/or chemotherapeutic agent or an anti-angiogenic agent or another anti-ErbB receptor antibody.

15

INTERNATIONAL SEARCH REPORT

International Application No

PC1/EP 02/04404

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K39/395 A61K31/00 C07K16/28 C07K16/46 A61P35/00
A61K38/00 //(A61K39/395, 31:00), (A61K39/395, 38:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, PAJ, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	WO 01 15730 A (GENENTECH INC) 8 March 2001 (2001-03-08) page 5, lines 28-30; page 6, lines 15-26; page 7, lines 17-21; page 8, lines 29-35; page 14, line 30-page 16, line 2; page 22, line 14-page 24, line 13; page 30, line 3-page 31, line 27	1-20
X	WO 00 69459 A (IMCLONE SYSTEMS INC) 23 November 2000 (2000-11-23) page 5, lines 11-20; page 12, line 23-page 13, line 13; page 19, line 9; page 21, first paragraph	1-4, 6, 10, 13-20

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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *G* document member of the same patent family

Date of the actual completion of the international search

21 August 2002

Date of mailing of the international search report

11/09/2002

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Renggli, J

INTERNATIONAL SEARCH REPORT

International Application No

101/EP 02/04404

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SOMASUNDARAM C ET AL: "DEVELOPMENT OF A TRISPECIFIC ANTIBODY CONJUGATE THAT DIRECTS TWO DISTINCT TUMOR-ASSOCIATED ANTIGENS TO CD64 ON MYELOID EFFECTOR CELLS" HUMAN ANTIBODIES, AMSTERDAM, NL, vol. 9, 1999, pages 47-54, XP000973177 ISSN: 1093-2607 the whole document	1-4,6, 8-20
X	EP 1 086 705 A (KYOWA HAKKO KOGYO KK) 28 March 2001 (2001-03-28) claims	5,20
X	GRUENDKER CARSTEN ET AL: "Luteinizing hormone-releasing hormone agonist triptorelin and antagonist cetorelix inhibit EGF-induced c-fos expression in human gynecological cancers." GYNECOLOGIC ONCOLOGY, vol. 78, no. 2, August 2000 (2000-08), pages 194-202, XP002210425 ISSN: 0090-8258 the whole document	12
X	BIANCO C ET AL: "ANTITUMOR ACTIVITY OF COMBINED TREATMENT OF HUMAN CANCER CELLS WITH IONIZING RADIATION AND ANTI-EPIDERMAL GROWTH FACTOR RECEPTOR MONOCLONAL ANTIBODY C225 PLUS TYPE I PROTEIN KINASE A ANTISENSE OLIGONUCLEOTIDE" CLINICAL CANCER RESEARCH, THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, no. 6, November 2000 (2000-11), pages 4343-4350, XP002951101 ISSN: 1078-0432 page 4348-4349, discussion	1-20
A	GLASS C K: "DIFFERENTIAL RECOGNITION OF TARGET GENES BY NUCLEAR RECEPTOR MONOMERS, DIMERS, AND HETERODIMERS" ENDOCRINE REVIEWS, BALTIMORE, MD, US, vol. 15, no. 3, 1 June 1994 (1994-06-01), pages 391-407, XP000572339 the whole document	1-20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 02/04404

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 18-20 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 02/04404

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
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			BR 0013814 A	23-04-2002
			EP 1210115 A1	05-06-2002
			TR 200200472 T2	21-06-2002
			WO 0115730 A1	08-03-2001
WO 0069459	A	23-11-2000	AU 4687100 A	05-12-2000
			BG 106110 A	30-04-2002
			BR 0010524 A	28-05-2002
			CN 1361700 T	31-07-2002
			EP 1218032 A1	03-07-2002
			NO 20015546 A	14-01-2002
			WO 0069459 A1	23-11-2000
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			CA 2328893 A1	25-11-1999
			EP 1086705 A1	28-03-2001
			WO 9959636 A1	25-11-1999

ANEXO 2



US 20060134064A1

(19) **United States**(12) **Patent Application Publication** (10) **Pub. No.: US 2006/0134064 A1**
Goldstein et al. (43) **Pub. Date: Jun. 22, 2006**(54) **COMBINED TREATMENT WITH
INTERFERON-ALPHA AND AN EPIDERMAL
GROWTH FACTOR RECEPTOR KINASE
INHIBITOR**(76) Inventors: **David Goldstein**, Sydney (AU);
Xian-Jun Qu, Jinan (CN); **Pamela J.**
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OSI PHARMACEUTICALS, INC.
41 PINELAWN ROAD
MELVILLE, NY 11747 (US)(21) Appl. No.: **11/303,728**(22) Filed: **Dec. 16, 2005****Related U.S. Application Data**(60) Provisional application No. 60/637,637, filed on Dec.
20, 2004.**Publication Classification**(51) **Int. Cl.****A61K 38/21** (2006.01)**A61K 31/175** (2006.01)**A61K 31/7048** (2006.01)(52) **U.S. Cl.** **424/85.7; 514/27; 514/589**

(57)

ABSTRACT

The present invention provides a method for treating tumors or tumor metastases in a patient, comprising administering to the patient simultaneously or sequentially a therapeutically effective amount of an EGFR kinase inhibitor and IFN α combination, with or without additional agents or treatments, such as other anti-cancer drugs or radiation therapy. The invention also encompasses a pharmaceutical composition that is comprised of an EGFR kinase inhibitor and IFN α combination in combination with a pharmaceutically acceptable carrier. A preferred example of an EGFR kinase inhibitor that can be used in practicing this invention is the compound erlotinib HCl (also known as TARCEVA®).

Figure 1

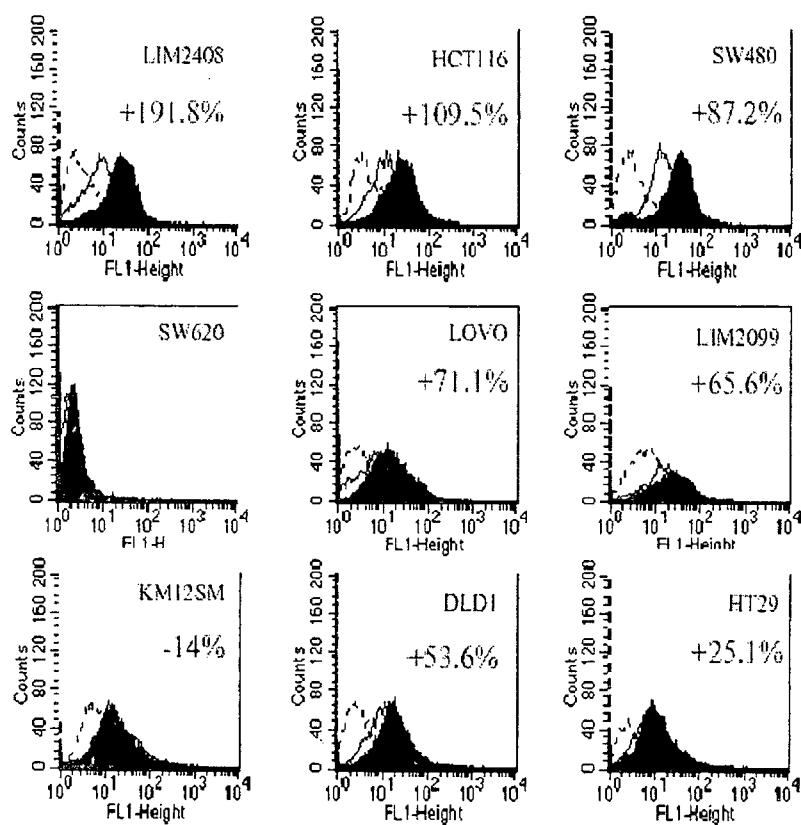


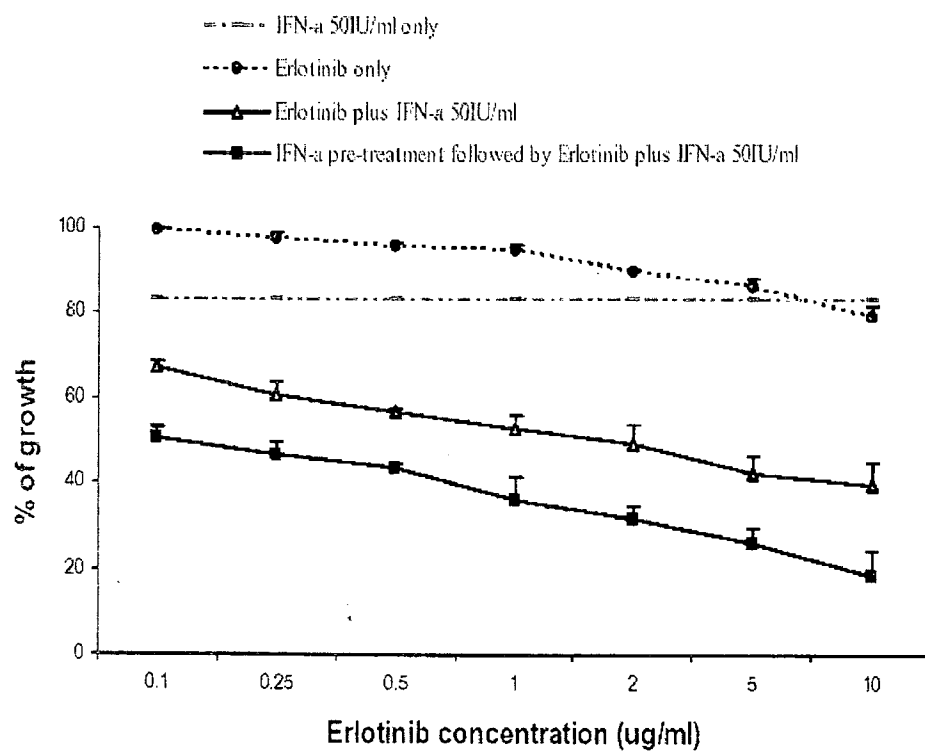
Figure 2 (a)

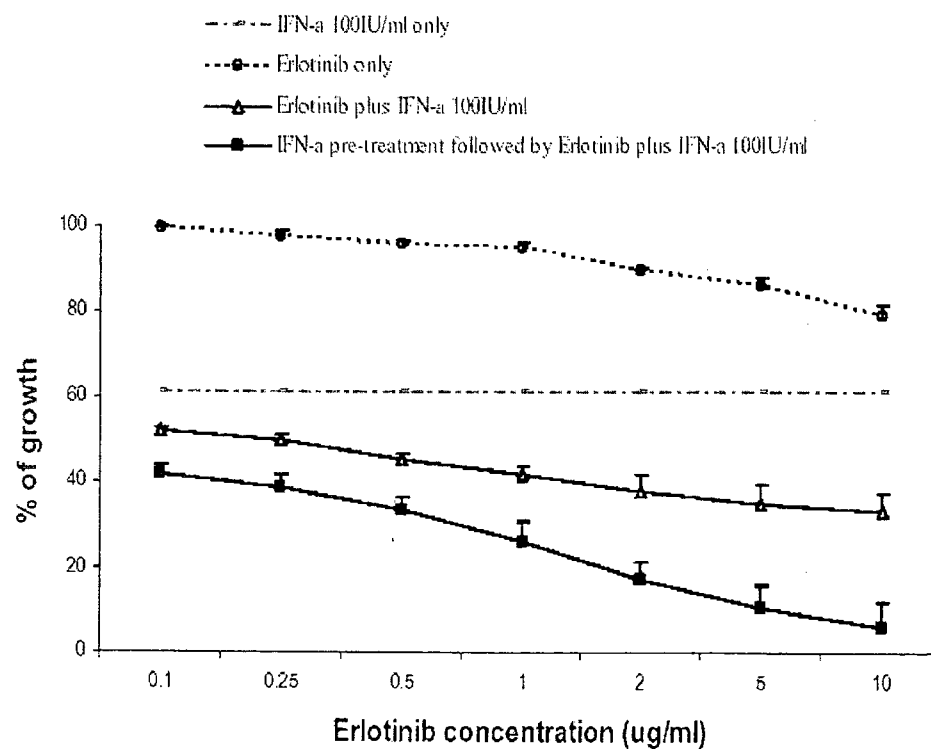
Figure 2 (b)

Figure 3

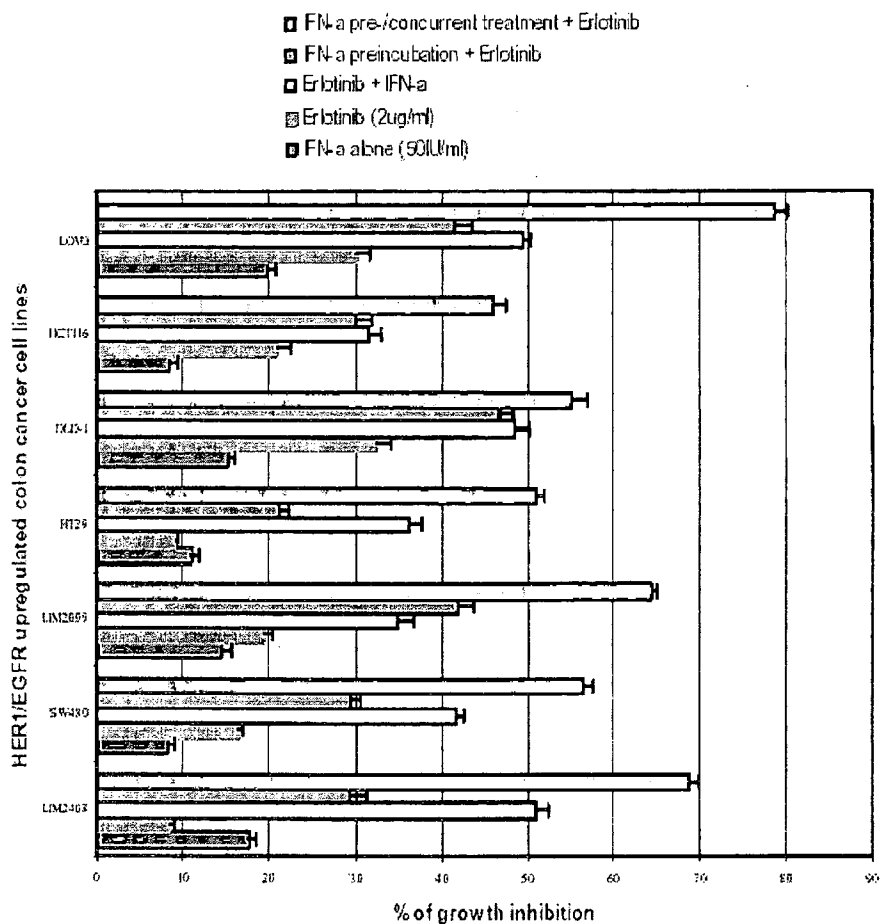


Figure 4Anti-proliferative effect of combining IFN- α with erlotinib versus IFN- α or erlotinib alone on human colon cancer cell lines

% of inhibition	Colon cancer cell lines								
	LIM2408 (n=3) ^a	SW480 (n=2)	LIM2099 (n=3)	HT29 (n=2)	DLD-1 (n=3)	HCT116 (n=3)	LOVO (n=3)	SW620 (n=2)	KM12SM (n=3)
IFN- α alone (50 IU/ml)	17.57 [1.01] ^b	8.16 [0.83]	14.48 [1.07]	10.84 [1.04]	15.22 [0.76]	8.44 [0.91]	19.67 [0.99]	12.53 [0.54]	9.33 [0.48]
Erlotinib (2 μ g/ml)	8.41 [0.59]	16.45 [0.56]	19.37 [1.01]	9.17 [0.28]	32.49 [1.7]	20.86 [1.53]	30.09 [1.5]	3.32 [0.55]	25.98 [1.33]
Erlotinib + IFN- α	51.05 [1.32]	41.66 [0.81]	34.91 [1.89]	36.12 [1.53]	48.45 [1.81]	31.42 [1.49]	49.49 [0.86]	15.23 [0.85]	26.67 [0.85]
^c Supra-additive	+25.07	+17.03	+1.06	+16.11	+0.74	+2.12	-0.27	-0.62	-8.64
95% confidence interval	21-29	13-20	-4-3	13-20	-6-5	-6-10	-4-4	-2-3	5-12
^d p value	<0.001	<0.001	0.845	<0.001	0.996	0.497	0.999	0.929	<0.001
^e Pr + Erlotinib	29.2 [2.01]	29.49 [1.05]	41.82 [1.89]	21.09 [1.23]	46.6 [1.58]	30.02 [1.72]	41.46 [2.01]	5.76 [0.76]	20.89 [0.99]
Supra-additive	+3.22	+4.86	+7.97	+1.08	-1.11	+0.72	-8.3	-10.09	-14.42
95% confidence interval	17-25	1.9-8	4-11	-5-3	-4-7	-14-3	5-12	8-13	11-18
p value	0.109	0.001	<0.001	0.923	0.954	0.279	<0.001	<0.001	<0.001
Pr + Erlotinib + IFN- α	68.72 [1.25]	56.41 [1.27]	64.33 [0.59]	50.89 [1.06]	55.13 [1.87]	46.04 [1.33]	78.83 [1.4]	14.63 [0.83]	20.38 [0.7]
Supra-additive	+42.74	+31.78	+30.48	+30.88	+7.42	+16.74	+29.07	-1.22	-14.93
95% confidence interval	13-21	28-35	27-34	27-34	2-13	8-25	25-33	-1-4	11-19
p value	<0.001	<0.001	<0.001	<0.001	0.007	<0.001	<0.001	0.546	<0.001

^an=number of experiments. ^bThe value in "[]" is the standard deviation of the mean percent inhibition. ^cAdditive was concluded if Erlotinib + IFN- α surpassed IFN- α -alone plus Erlotinib-alone. Supra-additive was concluded if the difference was statistically significant. ^dP values were analysed by a post-hoc Scheffe test after a significant Kruskal-Wallis analysis across three groups: [1] IFN- α -alone plus Erlotinib alone, [2] combined Erlotinib and IFN- α , [3] pre-treatment with IFN- α plus Erlotinib, and [4] pre-treatment with IFN- α plus combined Erlotinib and IFN- α . ^ePr means pre-treatment.

Figure 5Classification indexes of combining IFN- α with erlotinib versus IFN- α or erlotinib alone on human colon cancer cell lines

% of growth	Colon cancer cell lines								
	LIM2408 (n=3) ^a	SW480 (n=2)	LIM2099 (n=3)	HT29 (n=2)	DLD-1 (n=3)	HCT116 (n=3)	LOVO (n=3)	SW620 (n=2)	KM12SM (n=3)
IFN- α alone (50IU/ml)	82.43 [1.01] ^b	91.82 [0.83]	85.52 [1.07]	89.16 [1.04]	84.78 [0.76]	91.56 [0.91]	80.33 [0.99]	87.47 [0.54]	90.67 [0.48]
Erlotinib (2 μ g/ml)	91.59 [0.59]	83.55 [0.56]	80.63 [1.01]	90.83 [0.28]	67.51 [1.7]	79.14 [1.53]	69.91 [1.5]	96.68 [0.55]	74.02 [1.33]
Erlotinib + IFN- α	48.95 [1.32]	58.34 [0.81]	65.09 [1.89]	63.88 [1.53]	51.55 [1.81]	68.58 [1.49]	50.51 [0.86]	84.77 [0.85]	73.33 [0.85]
^c Classification index	0.6484	0.7605	0.9440	0.7883	0.9007	0.9454	0.8995	1.002	1.0926
^d Pr + Erlotinib	70.8 [2.01]	70.51 [1.05]	58.18 [1.89]	78.91 [1.23]	53.4 [1.58]	69.98 [1.72]	58.54 [2.01]	94.24 [0.76]	97.11 [0.99]
Classification index	0.9378	0.9191	0.8437	0.9744	0.9330	0.9657	1.0425	1.1144	1.4470
Pr + Erlotinib + IFN- α	31.28 [1.25]	43.58 [1.27]	35.67 [0.59]	49.11 [1.08]	44.87 [1.87]	53.96 [1.33]	21.17 [1.4]	85.37 [0.93]	79.62 [0.7]
Classification index	0.4143	0.5682	0.5173	0.6084	0.7840	0.7446	0.3788	1.0166	1.1864

^an=number of experiments. ^bThe value in "[]" is the standard deviation of the mean percent inhibition. ^cClassification index: <0.9=supra-additivity, 0.9-1=additivity, >1=subadditivity. ^dPr means pre-treatment.

COMBINED TREATMENT WITH INTERFERON-ALPHA AND AN EPIDERMAL GROWTH FACTOR RECEPTOR KINASE INHIBITOR

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 60/637,637, filed Dec. 20, 2004, which is herein incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] The present invention is directed to compositions and methods for treating cancer patients. In particular, the present invention is directed to combined treatment of patients with interferon- α (IFN α) and an epidermal growth factor receptor (EGFR) kinase inhibitor.

[0003] Cancer is a generic name for a wide range of cellular malignancies characterized by unregulated growth, lack of differentiation, and the ability to invade local tissues and metastasize. These neoplastic malignancies affect, with various degrees of prevalence, every tissue and organ in the body.

[0004] A multitude of therapeutic agents have been developed over the past few decades for the treatment of various types of cancer. The most commonly used types of anticancer agents include: DNA-alkylating agents (e.g., cyclophosphamide, ifosfamide), antimetabolites (e.g., methotrexate, a folate antagonist, and 5-fluorouracil, a pyrimidine antagonist), microtubule disrupters (e.g., vincristine, vinblastine, paclitaxel), DNA intercalators (e.g., doxorubicin, daunomycin, cisplatin), and hormone therapy (e.g., tamoxifen, flutamide).

[0005] Colorectal cancer is among the leading causes of cancer-related morbidity and mortality in the U.S. Treatment of this cancer depends largely on the size, location and stage of the tumor, whether the malignancy has spread to other parts of the body (metastasis), and on the patient's general state of health. Options include surgical removal of tumors for early stage localized disease, chemotherapy and radiotherapy. However, chemotherapy is currently the only treatment for metastatic disease. 5-fluorouracil is currently the most effective single-agent treatment for advanced colorectal cancer, with response rates of about 10%. Additionally, new agents such as the topoisomerase I inhibitor irinotecan (CPT11), the platinum-based cytotoxic agent oxaliplatin (e.g. ELOXATIN™), and the EGFR kinase inhibitor erlotinib ([6,7-bis(2-methoxyethoxy)-4-quinazolin-4-yl]-(3-ethynylphenyl)amine, e.g. erlotinib HCl, TARCEVA®) have shown promise in treatment.

[0006] Over-expression of the epidermal growth factor receptor (EGFR) kinase, or its ligand TGF- α , is frequently associated with many cancers, including breast, lung, colorectal, bladder, and head and neck cancers (Salomon D. S., et al. (1995) *Crit. Rev. Oncol. Hematol.* 19:183-232; Wells, A. (2000) *Signal*, 1:4-11), and is believed to contribute to the malignant growth of these tumors. A specific deletion-mutation in the EGFR gene has also been found to increase cellular tumorigenicity (Halatsch, M-E. et al. (2000) *J. Neurosurg.* 92:297-305; Archer, G. E. et al. (1999) *Clin. Cancer Res.* 5:2646-2652).

Activation of EGFR stimulated signaling pathways promote multiple processes that are potentially cancer-promoting, e.g. proliferation, angiogenesis, cell motility and invasion, decreased apoptosis and induction of drug resistance. The development for use as anti-tumor agents of compounds that directly inhibit the kinase activity of the EGFR, as well as antibodies that reduce EGFR kinase activity by blocking EGFR activation, are areas of intense research effort (de Bono J. S. and Rowinsky, E. K. (2002) *Trends in Mol. Medicine* 8:S19-S26; Dancey, J. and Sausville, E. A. (2003) *Nature Rev. Drug Discovery* 2:92-313). Several studies have demonstrated or disclosed that some EGFR kinase inhibitors can improve tumor cell or neoplasia killing when used in combination with certain other anti-cancer or chemotherapeutic agents or treatments (e.g. Raben, D. et al. (2002) *Semin. Oncol.* 29:37-46; Herbst, R. S. et al. (2001) *Expert Opin. Biol. Ther.* 1:719-732; Magne, N et al. (2003) *Clin. Can. Res.* 9:4735-4732; Magne, N. et al. (2002) *British Journal of Cancer* 86:819-827; Torrance, C. J. et al. (2000) *Nature Med.* 6:1024-1028; Gupta, R. A. and DuBois, R. N. (2000) *Nature Med.* 6:974-975; Tortora, et al. (2003) *Clin. Cancer Res.* 9:1566-1572; Solomon, B. et al (2003) *Int. J. Radiat. Oncol. Biol. Phys.* 55:713-723; Krishnan, S. et al. (2003) *Frontiers in Bioscience* 8, e1-13; Huang, S et al. (1999) *Cancer Res.* 59:1935-1940; Contessa, J. N. et al. (1999) *Clin. Cancer Res.* 5:405-411; Li, M. et al. *Clin. (2002) Cancer Res.* 8:3570-3578; Ciardiello, F. et al. (2003) *Clin. Cancer Res.* 9:1546-1556; Ciardiello, F. et al. (2000) *Clin. Cancer Res.* 6:3739-3747; Grunwald, V. and Hidalgo, M. (2003) *J. Nat. Cancer Inst.* 95:851-867; Seymour L. (2003) *Current Opin. Investig. Drugs* 4(6):658-666; Khalil, M. Y. et al. (2003) *Expert Rev. Anticancer Ther.* 3:367-380; Bulgaru, A. M. et al. (2003) *Expert Rev. Anticancer Ther.* 3:269-279; Dancey, J. and Sausville, E. A. (2003) *Nature Rev. Drug Discovery* 2:92-313; Kim, E. S. et al. (2001) *Current Opinion Oncol.* 13:506-513; Arteaga, C. L. and Johnson, D. H. (2001) *Current Opinion Oncol.* 13:491-498; Ciardiello, F. et al. (2000) *Clin. Cancer Res.* 6:2053-2063; Patent Publication Nos: US 2003/0108545; US 2002/0076408; and US 2003/0157104; and International Patent Publication Nos: WO 99/60023; WO 01/12227; WO 02/055106; WO 03/088971; WO 01/34574; WO 01/76586; WO 02/05791; and WO 02/089842).

[0007] An anti-neoplastic drug would ideally kill cancer cells selectively, with a wide therapeutic index relative to its toxicity towards non-malignant cells. It would also retain its efficacy against malignant cells, even after prolonged exposure to the drug. Unfortunately, none of the current chemotherapies possess such an ideal profile. Instead, most possess very narrow therapeutic indexes. Furthermore, cancerous cells exposed to slightly sub-lethal concentrations of a chemotherapeutic agent will very often develop resistance to such an agent, and quite often cross-resistance to several other antineoplastic agents as well.

[0008] Thus, there is a need for more efficacious treatment for neoplasia and other proliferative disorders. Strategies for enhancing the therapeutic efficacy of existing drugs have involved changes in the schedule for their administration, and also their use in combination with other anticancer or biochemical modulating agents. Combination therapy is well known as a method that can result in greater efficacy and diminished side effects relative to the use of the therapeutically relevant dose of each agent alone. In some cases, the efficacy of the drug combination is additive (the efficacy

of the combination is approximately equal to the sum of the effects of each drug alone), but in other cases the effect is synergistic (the efficacy of the combination is greater than the sum of the effects of each drug given alone). For example, when combined with 5-FU and leucovorin, oxaliplatin exhibits response rates of 25-40% as first-line treatment for colorectal cancer (Raymond, E. et al. (1998) *Semin Oncol.* 25(2 Suppl. 5):4-12).

[0009] However, there remains a critical need for improved treatments for colorectal and other cancers. This invention provides anti-cancer combination therapies that reduce the dosages for individual components required for efficacy, thereby decreasing side effects associated with each agent, while maintaining or increasing therapeutic value. The invention described herein provides new drug combinations, and methods for using drug combinations in the treatment of colorectal and other cancers.

SUMMARY OF THE INVENTION

[0010] The present invention provides a method for treating tumors or tumor metastases in a patient, comprising administering to the patient simultaneously or sequentially a therapeutically effective amount of an EGFR kinase inhibitor and IFN α combination, with or without additional agents or treatments, such as other anti-cancer drugs or radiation therapy.

[0011] The invention also encompasses a pharmaceutical composition that is comprised of an EGFR kinase inhibitor and IFN α combination in combination with a pharmaceutically acceptable carrier.

[0012] A preferred example of an EGFR kinase inhibitor that can be used in practicing this invention is the compound erlotinib HCl (also known as TARCEVA®).

BRIEF DESCRIPTION OF THE FIGURES

[0013] **FIG. 1:** Cell-surface HER1/EGFR expression measured by flow cytometry in representative cell samples at 72 h (for LOVO cells measurement was at 24 h) of culture following IFN α treatment. Untreated cells using isotype-matched irrelevant antibody (dotted curve) or anti-HER1/EGFR antibody alone (thin black curve) are shown together with the relevant IFN α (100 IU/mL)-treated cells (black area) for each cell line. Percent change in HER1/EGFR expression by treated cells versus relevant untreated controls are shown.

[0014] **FIG. 2:** Anti-proliferative effect of different doses of interferon-alpha [(a) 50 IU/mL and (b) 100 IU/mL] concurrent-treatment and/or pre-treatment with different doses of erlotinib on LIM2408 cells by crystal violet colorimetric assay. Error bars show the variation in mean percent growth of all replicates in duplicate experiments.

[0015] **FIG. 3:** Supra-additive/additive effect of interferon-alpha pre- and/or concurrent-treatment with erlotinib on seven HER1/EGFR upregulated colon cancer cell lines. Significant differences exist across the four groups (erlotinib-alone plus IFN-alpha-alone as one group) of individual cell lines by the non-parametric Kniskal-Wallis test and between supra-additive group and that of erlotinib-alone plus IFN-alpha-alone (Scheffe, $p < 0.001$). Error bars show the variation in mean percent growth inhibition of all repeated experiments.

[0016] **FIG. 4:** Anti-proliferative effect of combining IFN- α with erlotinib versus IFN- α or erlotinib alone on human colon cancer cell lines.

[0017] **FIG. 5:** Classification indexes of combining IFN- α with erlotinib versus IFN- α or erlotinib alone on human colon cancer cell lines.

DETAILED DESCRIPTION OF THE INVENTION

[0018] The term "cancer" in an animal refers to the presence of cells possessing characteristics typical of cancer-causing cells, such as uncontrolled proliferation, immortality, metastatic potential, rapid growth and proliferation rate, and certain characteristic morphological features. Often, cancer cells will be in the form of a tumor, but such cells may exist alone within an animal, or may circulate in the blood stream as independent cells, such as leukemic cells.

[0019] "Abnormal cell growth", as used herein, unless otherwise indicated, refers to cell growth that is independent of normal regulatory mechanisms (e.g., loss of contact inhibition). This includes the abnormal growth of: (1) tumor cells (tumors) that proliferate by expressing a mutated tyrosine kinase or overexpression of a receptor tyrosine kinase; (2) benign and malignant cells of other proliferative diseases in which aberrant tyrosine kinase activation occurs; (4) any tumors that proliferate by receptor tyrosine kinases; (5) any tumors that proliferate by aberrant serine/threonine kinase activation; and (6) benign and malignant cells of other proliferative diseases in which aberrant serine/threonine kinase activation occurs.

[0020] The term "treating" as used herein, unless otherwise indicated, means reversing, alleviating, inhibiting the progress of, or preventing, either partially or completely, the growth of tumors, tumor metastases, or other cancer-causing or neoplastic cells in a patient. The term "treatment" as used herein, unless otherwise indicated, refers to the act of treating.

[0021] The phrase "a method of treating" or its equivalent, when applied to, for example, cancer refers to a procedure or course of action that is designed to reduce or eliminate the number of cancer cells in an animal, or to alleviate the symptoms of a cancer. "A method of treating" cancer or another proliferative disorder does not necessarily mean that the cancer cells or other disorder will, in fact, be eliminated, that the number of cells or disorder will, in fact, be reduced, or that the symptoms of a cancer or other disorder will, in fact, be alleviated. Often, a method of treating cancer will be performed even with a low likelihood of success, but which, given the medical history and estimated survival expectancy of an animal, is nevertheless deemed an overall beneficial course of action.

[0022] The term "therapeutically effective agent" means a composition that will elicit the biological or medical response of a tissue, system, animal or human that is being sought by the researcher, veterinarian, medical doctor or other clinician.

[0023] The term "therapeutically effective amount" or "effective amount" means the amount of the subject compound or combination that will elicit the biological or

medical response of a tissue, system, animal or human that is being sought by the researcher, veterinarian, medical doctor or other clinician.

[0024] The data presented in the Examples herein below demonstrate that co-administration of IFN α with an EGFR kinase inhibitor is effective for treatment of patients with advanced cancers, such as colorectal cancer or bladder cancer. Accordingly, the present invention provides a method for treating tumors or tumor metastases in a patient, including colorectal cancer or bladder cancer tumors, comprising administering to said patient simultaneously or sequentially a therapeutically effective amount of an EGFR kinase inhibitor and IFN α combination. In one embodiment of this method, IFN α is administered prior to the EGFR kinase inhibitor. In another embodiment of this method, IFN α is pre-administered prior to administration of a combination of EGFR kinase inhibitor and IFN α .

[0025] The present invention further provides a method for treating tumors or tumor metastases in a patient, comprising administering to the patient simultaneously or sequentially a therapeutically effective amount of an EGFR kinase inhibitor and IFN α combination, and in addition, one or more other cytotoxic, chemotherapeutic or anti-cancer agents, or compounds that enhance the effects of such agents.

[0026] In the context of this invention, additional other cytotoxic, chemotherapeutic or anti-cancer agents, or compounds that enhance the effects of such agents, include, for example: alkylating agents or agents with an alkylating action, such as cyclophosphamide (CTX; e.g. CYTOXAN®), chlorambucil (CHL; e.g. LEUKERAN®), cisplatin (CisP; e.g. PLATINOL®), busulfan (e.g. MYLE-RAN®), melphalan, carmustine (BCNU), streptozotocin, triethylenemelamine (TEM), mitomycin C, and the like; anti-metabolites, such as methotrexate (MTX), etoposide (VP16; e.g. VEPESID®), 6-mercaptopurine (6MP), 6-thioguanine (6TG), cytarabine (Ara-C), 5-fluorouracil (5-FU), capecitabine (e.g. XELODA®), dacarbazine (DTIC), and the like; antibiotics, such as actinomycin D, doxorubicin (DXR; e.g. ADRIAMYCIN®), daunorubicin (daunomycin), bleomycin, mithramycin and the like; alkaloids, such as vinca alkaloids such as vincristine (VCR), vinblastine, and the like; and other antitumor agents, such as paclitaxel (e.g. TAXOL®) and paclitaxel derivatives, the cytostatic agents, glucocorticoids such as dexamethasone (DEX; e.g. DEC-ADRON®) and corticosteroids such as prednisone, nucleoside enzyme inhibitors such as hydroxyurea, amino acid depleting enzymes such as asparaginase, leucovorin and other folic acid derivatives, and similar, diverse antitumor agents. The following agents may also be used as additional agents: amifostine (e.g. ETHYOL®), dactinomycin, mechlorethamine (nitrogen mustard), streptozocin, cyclophosphamide, lomustine (CCNU), doxorubicin lipo (e.g. DOXIL®), gemcitabine (e.g. GEMZAR®), daunorubicin lipo (e.g. DAUNOXOME®), procarbazine, mitomycin, docetaxel (e.g. TAXOTERE®), aldesleukin, carboplatin, oxaliplatin, cladribine, camptothecin, CPT 11 (irinotecan), 10-hydroxy 7-ethyl-camptothecin (SN38), floxuridine, fludarabine, ifosfamide, idarubicin, mesna, interferon beta, mitoxantrone, topotecan, leuprolide, megestrol, melphalan, mercaptopurine, plicamycin, mitotane, pegaspargase, pen-

tostatin, pipobroman, plicamycin, tamoxifen, teniposide, testolactone, thioguanine, thiotepa, uracil mustard, vinorelbine, chlorambucil.

[0027] The present invention further provides a method for treating tumors or tumor metastases in a patient, comprising administering to said patient simultaneously or sequentially a therapeutically effective amount of an EGFR kinase inhibitor and IFN α combination, and in addition, one or more anti-hormonal agents. As used herein, the term "anti-hormonal agent" includes natural or synthetic organic or peptidic compounds that act to regulate or inhibit hormone action on tumors.

[0028] Antihormonal agents include, for example: steroid receptor antagonists, anti-estrogens such as tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, other aromatase inhibitors, 42-hydroxytamoxifen, trioxifene, keoxifene, LY 117018, onapristone, and toremifene (e.g. FARESTON®); anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above; agonists and/or antagonists of glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH) and LHRH (luteinizing hormone-releasing hormone); the LHRH agonist goserelin acetate, commercially available as ZOLADEX® (AstraZeneca); the LHRH antagonist D-alaninamide N-acetyl-3-(2-naphthalenyl)-D-alanyl-4-chloro-D-phenylalanyl-3-(3-pyridinyl)-D-alanyl-L-seryl-N6-(3-pyridinylcarbonyl)-L-lysyl-N6-(3-pyridinylcarbonyl)-D-lysyl-L-leucyl-N6-(1-methylethyl)-L-lysyl-L-proline (e.g. ANTIDE®, Ares-Serono); the LHRH antagonist ganirelix acetate; the steroidal anti-androgens cyproterone acetate (CPA) and megestrol acetate, commercially available as MEGACE® (Bristol-Myers Oncology); the nonsteroidal anti-androgen flutamide (2-methyl-N-[4,20-nitro-3-(trifluoromethyl)phenyl]propanamide), commercially available as EULEXIN® (Schering Corp.); the non-steroidal anti-androgen nilutamide, (5,5-dimethyl-3-[4-nitro-3-(trifluoromethyl)-4'-nitrophenyl]-4,4-dimethyl-imidazolidine-dione); and antagonists for other non-permissive receptors, such as antagonists for RAR, RXR, TR, VDR, and the like.

[0029] The use of the cytotoxic and other anticancer agents described above in chemotherapeutic regimens is generally well characterized in the cancer therapy arts, and their use herein falls under the same considerations for monitoring tolerance and effectiveness and for controlling administration routes and dosages, with some adjustments. For example, the actual dosages of the cytotoxic agents may vary depending upon the patient's cultured cell response determined by using histoculture methods. Generally, the dosage will be reduced compared to the amount used in the absence of additional other agents.

[0030] Typical dosages of an effective cytotoxic agent can be in the ranges recommended by the manufacturer, and where indicated by in vitro responses or responses in animal models, can be reduced by up to about one order of magnitude concentration or amount. Thus, the actual dosage will depend upon the judgment of the physician, the condition of the patient, and the effectiveness of the therapeutic method based on the in vitro responsiveness of the primary cultured malignant cells or histocultured tissue sample, or the responses observed in the appropriate animal models.

[0031] The present invention further provides a method for treating tumors or tumor metastases in a patient, comprising administering to said patient simultaneously or sequentially a therapeutically effective amount of an EGFR kinase inhibitor and IFN α combination, and in addition one or more angiogenesis inhibitors.

[0032] Anti-angiogenic agents include, for example: VEGFR inhibitors, such as SU-5416 and SU-6668 (Sugen Inc. of South San Francisco, Calif., USA), or as described in, for example International Application Nos. WO 99/24440, WO 99/62890, WO 95/21613, WO 99/61422, WO 98/50356, WO 99/10349, WO 97/32856, WO 97/22596, WO 98/54093, WO 98/02438, WO 99/16755, and WO 98/02437, and U.S. Pat. Nos. 5,883,113, 5,886,020, 5,792,783, 5,834,504 and 6,235,764; VEGF inhibitors such as IM862 (Cytran Inc. of Kirkland, Wash., USA); angiozyme, a synthetic ribozyme from Ribozyme (Boulder, Colo.) and Chiron (Emeryville, Calif.); and antibodies to VEGF, such as bevacizumab (e.g. AVASTINTM, Genentech, South San Francisco, Calif.), a recombinant humanized antibody to VEGF; integrin receptor antagonists and integrin antagonists, such as to $\alpha_v\beta_3$, $\alpha_v\beta_5$ and $\alpha_v\beta_6$ integrins, and subtypes thereof, e.g. cilengitide (EMD 121974), or the anti-integrin antibodies, such as for example $\alpha_v\beta_3$ specific humanized antibodies (e.g. VITAXIN[®]); factors such as IFN- α (U.S. Pat. Nos. 4,153,901, 4,503,035, and 5,231,176); angiostatin and plasminogen fragments (e.g. kringle 1-4, kringle 5, kringle 1-3 (O'Reilly, M. S. et al. (1994) Cell 79:315-328; Cao et al. (1996) J. Biol. Chem. 271: 29461-29467; Cao et al. (1997) J. Biol. Chem. 272:22924-22928); endostatin (O'Reilly, M. S. et al. (1997) Cell 88:277; and International Patent Publication No. WO 97/15666); thrombospondin (TSP-1; Frazier, (1991) Curr. Opin. Cell Biol. 3:792); platelet factor 4 (PF4); plasminogen activator/urokinase inhibitors; urokinase receptor antagonists; heparinases; fumagillin analogs such as TNP-4701; suramin and suramin analogs; angiostatic steroids; bFGF antagonists; flk-1 and flt-1 antagonists; anti-angiogenesis agents such as MMP-2 (matrix-metalloproteinase 2) inhibitors and MMP-9 (matrix-metalloproteinase 9) inhibitors. Examples of useful matrix metalloproteinase inhibitors are described in International Patent Publication Nos. WO 96/33172, WO 96/27583, WO 98/07697, WO 98/03516, WO 98/34918, WO 98/34915, WO 98/33768, WO 98/30566, WO 90/05719, WO 99/52910, WO 99/52889, WO 99/29667, and WO 99/07675, European Patent Publication Nos. 818,442, 780,386, 1,004,578, 606,046, and 931,788; Great Britain Patent Publication No. 9912961, and U.S. Pat. Nos. 5,863,949 and 5,861,510. Preferred MMP-2 and MMP-9 inhibitors are those that have little or no activity inhibiting MMP-1. More preferred, are those that selectively inhibit MMP-2 and/or MMP-9 relative to the other matrix-metalloproteinases (i.e. MMP-1, MMP-3, MMP-4, MMP-5, MMP-6, MMP-7, MMP-8, MMP-10, MMP-11, MMP-12, and MMP-13).

[0033] The present invention further provides a method for treating tumors or tumor metastases in a patient, comprising administering to the patient simultaneously or sequentially a therapeutically effective amount of an EGFR kinase inhibitor and IFN α combination, and in addition one or more tumor cell pro-apoptotic or apoptosis-stimulating agents.

[0034] The present invention further provides a method for treating tumors or tumor metastases in a patient, com-

prising administering to said patient simultaneously or sequentially a therapeutically effective amount of an EGFR kinase inhibitor and IFN α combination, and in addition one or more signal transduction inhibitors.

[0035] Signal transduction inhibitors include, for example: erbB2 receptor inhibitors, such as organic molecules, or antibodies that bind to the erbB2 receptor, for example, trastuzumab (e.g. HERCEPTIN[®]); inhibitors of other protein tyrosine-kinases, e.g. imatinib (e.g. GLEEVEC[®]); ras inhibitors; raf inhibitors; MEK inhibitors; mTOR inhibitors; cyclin dependent kinase inhibitors; protein kinase C inhibitors; and PDK-1 inhibitors (see Dancsey, J. and Sausville, E. A. (2003) Nature Rev. Drug Discovery 2:92-313, for a description of several examples of such inhibitors, and their use in clinical trials for the treatment of cancer).

[0036] ErbB2 receptor inhibitors include, for example: ErbB2 receptor inhibitors, such as GW-282974 (Glaxo Wellcome plc), monoclonal antibodies such as AR-209 (Aronex Pharmaceuticals Inc. of The Woodlands, Tex., USA) and 2B-1 (Chiron), and erbB2 inhibitors such as those described in International Publication Nos. WO 98/02434, WO 99/35146, WO 99/35132, WO 98/02437, WO 97/13760, and WO 95/19970, and U.S. Pat. Nos. 5,587,458, 5,877,305, 6,465,449 and 6,541,481.

[0037] The present invention further thus provides a method for treating tumors or tumor metastases in a patient, comprising administering to said patient simultaneously or sequentially a therapeutically effective amount of an EGFR kinase inhibitor and IFN α combination, and in addition an anti-HER2 antibody or an immunotherapeutically active fragment thereof.

[0038] The present invention further provides a method for treating tumors or tumor metastases in a patient, comprising administering to said patient simultaneously or sequentially a therapeutically effective amount of an EGFR kinase inhibitor and IFN α combination, and in addition one or more additional anti-proliferative agents.

[0039] Additional antiproliferative agents include, for example: Inhibitors of the enzyme farnesyl protein transferase and inhibitors of the receptor tyrosine kinase PDGFR, including the compounds disclosed and claimed in U.S. Pat. Nos. 6,080,769, 6,194,438, 6,258,824, 6,586,447, 6,071,935, 6,495,564, 6,150,377, 6,596,735 and 6,479,513, and International Patent Publication WO 01/40217.

[0040] The present invention further provides a method for treating tumors or tumor metastases in a patient, comprising administering to the patient simultaneously or sequentially a therapeutically effective amount of an EGFR kinase inhibitor and IFN α combination, and in addition a COX II (cyclooxygenase II) inhibitor. Examples of useful COX-II inhibitors include alecoxib (e.g. CELEBREXTM), valdecoxib, and rofecoxib.

[0041] The present invention further provides a method for treating tumors or tumor metastases in a patient, comprising administering to the patient simultaneously or sequentially a therapeutically effective amount of an EGFR kinase inhibitor and IFN α combination, and in addition treatment with radiation or a radiopharmaceutical.

[0042] The source of radiation can be either external or internal to the patient being treated. When the source is

external to the patient, the therapy is known as external beam radiation therapy (EBRT). When the source of radiation is internal to the patient, the treatment is called brachytherapy (BT). Radioactive atoms for use in the context of this invention can be selected from the group including, but not limited to, radium, cesium-137, iridium-192, americium-241, gold-198, cobalt-57, copper-67, technetium-99, iodine-123, iodine-131, and indium-111. Where the EGFR kinase inhibitor according to this invention is an antibody, it is also possible to label the antibody with such radioactive isotopes.

[0043] Radiation therapy is a standard treatment for controlling unresectable or inoperable tumors and/or tumor metastases. Improved results have been seen when radiation therapy has been combined with chemotherapy. Radiation therapy is based on the principle that high-dose radiation delivered to a target area will result in the death of reproductive cells in both tumor and normal tissues. The radiation dosage regimen is generally defined in terms of radiation absorbed dose (Gy), time and fractionation, and must be carefully defined by the oncologist. The amount of radiation a patient receives will depend on various considerations, but the two most important are the location of the tumor in relation to other critical structures or organs of the body, and the extent to which the tumor has spread. A typical course of treatment for a patient undergoing radiation therapy will be a treatment schedule over a 1 to 6 week period, with a total dose of between 10 and 80 Gy administered to the patient in a single daily fraction of about 1.8 to 2.0 Gy, 5 days a week. In a preferred embodiment of this invention there is synergy when tumors in human patients are treated with the combination treatment of the invention and radiation. In other words, the inhibition of tumor growth by means of the agents comprising the combination of the invention is enhanced when combined with radiation, optionally with additional chemotherapeutic or anticancer agents. Parameters of adjuvant radiation therapies are, for example, contained in International Patent Publication WO 99/60023.

[0044] The present invention further provides a method for treating tumors or tumor metastases in a patient, comprising administering to the patient simultaneously or sequentially a therapeutically effective amount of an EGFR kinase inhibitor and IFN α combination, and in addition treatment with one or more agents capable of enhancing antitumor immune responses.

[0045] Agents capable of enhancing antitumor immune responses include, for example: CTLA4 (cytotoxic lymphocyte antigen 4) antibodies (e.g. MDX-CTLA4), and other agents capable of blocking CTLA4. Specific CTLA4 antibodies that can be used in the present invention include those described in U.S. Pat. No. 6,682,736.

[0046] The present invention further provides a method for reducing the side effects caused by the treatment of tumors or tumor metastases in a patient with an EGFR kinase inhibitor or IFN, comprising administering to the patient simultaneously or sequentially a therapeutically effective amount of an EGFR kinase inhibitor and IFN α combination, in amounts that are effective to produce an additive, or a superadditive or synergistic antitumor effect, and that are effective at inhibiting the growth of the tumor.

[0047] The present invention further provides a method for the treatment of cancer, comprising administering to a subject in need of such treatment (i) an effective first amount

of an EGFR kinase inhibitor, or a pharmaceutically acceptable salt thereof; and (ii) an effective second amount of IFN α . In this method the cancer can be any of those referred to herein below, including colorectal or bladder cancers.

[0048] The present invention also provides a method for the treatment of cancer, comprising administering to a subject in need of such treatment (i) a sub-therapeutic first amount of an EGFR kinase inhibitor, or a pharmaceutically acceptable salt thereof; and (ii) a sub-therapeutic second amount of IFN α . In this method the cancer can be any of those referred to herein below, including colorectal or bladder cancers.

[0049] The present invention also provides a method for the treatment of cancer, comprising administering to a subject in need of such treatment (i) an effective first amount of an EGFR kinase inhibitor, or a pharmaceutically acceptable salt thereof; and (ii) a sub-therapeutic second amount of IFN α . In this method the cancer can be any of those referred to herein below, including colorectal or bladder cancers.

[0050] The present invention also provides a method for the treatment of cancer, comprising administering to a subject in need of such treatment (i) a sub-therapeutic first amount of an EGFR kinase inhibitor, or a pharmaceutically acceptable salt thereof; and (ii) an effective second amount of IFN α . In this method the cancer can be any of those referred to herein below, including colorectal or bladder cancers.

[0051] In the preceding methods the order of administration of the first and second amounts can be simultaneous or sequential, i.e. IFN α can be administered before the EGFR kinase inhibitor, after the EGFR inhibitor, or at the same time as the EGFR kinase inhibitor. In a preferred embodiment, IFN α is administered prior to the EGFR kinase inhibitor, or a combination of the EGFR kinase inhibitor and IFN α .

[0052] In the context of this invention, an "effective amount" of an agent or therapy is as defined above. A "sub-therapeutic amount" of an agent or therapy is an amount less than the effective amount for that agent or therapy, but when combined with an effective or sub-therapeutic amount of another agent or therapy can produce a result desired by the physician, due to, for example, synergy in the resulting efficacious effects, or reduced side effects.

[0053] Additionally, the present invention provides a pharmaceutical composition comprising an EGFR inhibitor and IFN α in a pharmaceutically acceptable carrier.

[0054] As used herein, the term "patient" preferably refers to a human in need of treatment with an EGFR kinase inhibitor for any purpose, and more preferably a human in need of such a treatment to treat cancer, or a precancerous condition or lesion. However, the term "patient" can also refer to non-human animals, preferably mammals such as dogs, cats, horses, cows, pigs, sheep and non-human primates, among others, that are in need of treatment with an EGFR kinase inhibitor.

[0055] In a preferred embodiment, the patient is a human in need of treatment for cancer, or a precancerous condition or lesion. The cancer is preferably any cancer treatable, either partially or completely, by administration of an EGFR

kinase inhibitor. The cancer may be, for example, lung cancer, non small cell lung (NSCL) cancer, bronchioloalveolar cell lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, gastric cancer, colon cancer, breast cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, prostate cancer, cancer of the bladder, cancer of the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis, mesothelioma, hepatocellular cancer, biliary cancer, chronic or acute leukemia, lymphocytic lymphomas, neoplasms of the central nervous system (CNS), spinal axis tumors, brain stem glioma, glioblastoma multiforme, astrocytomas, schwannomas, ependymomas, medulloblastomas, meningiomas, squamous cell carcinomas, pituitary adenomas, including refractory versions of any of the above cancers, or a combination of one or more of the above cancers. The precancerous condition or lesion includes, for example, the group consisting of oral leukoplakia, actinic keratosis (solar keratosis), precancerous polyps of the colon or rectum, gastric epithelial dysplasia, adenomatous dysplasia, hereditary nonpolyposis colon cancer syndrome (HNPCC), Barrett's esophagus, bladder dysplasia, and precancerous cervical conditions.

[0056] The term "refractory" as used herein is used to define a cancer for which treatment (e.g. chemotherapy drugs, biological agents, and/or radiation therapy) has proven to be ineffective. A refractory cancer tumor may shrink, but not to the point where the treatment is determined to be effective. Typically however, the tumor stays the same size as it was before treatment (stable disease), or it grows (progressive disease).

[0057] For purposes of the present invention, "co-administration of" and "co-administering" IFN α with an EGFR kinase inhibitor (both components referred to hereinafter as the "two active agents") refer to any administration of the two active agents, either separately or together, where the two active agents are administered as part of an appropriate dose regimen designed to obtain the benefit of the combination therapy. Thus, the two active agents can be administered either as part of the same pharmaceutical composition or in separate pharmaceutical compositions. IFN α can be administered prior to, at the same time as, or subsequent to administration of the EGFR kinase inhibitor, or in some combination thereof. Where the EGFR kinase inhibitor is administered to the patient at repeated intervals, e.g., during a standard course of treatment, IFN α can be administered prior to, at the same time as, or subsequent to, each administration of the EGFR kinase inhibitor, or some combination thereof, or at different intervals in relation to the EGFR kinase inhibitor treatment, or in a single dose prior to, at any time during, or subsequent to the course of treatment with the EGFR kinase inhibitor.

[0058] The EGFR kinase inhibitor will typically be administered to the patient in a dose regimen that provides for the most effective treatment of the cancer (from both efficacy and safety perspectives) for which the patient is being

treated, as known in the art, and as disclosed, e.g. in International Patent Publication No. WO 01/34574. In conducting the treatment method of the present invention, the EGFR kinase inhibitor can be administered in any effective manner known in the art, such as by oral, topical, intravenous, intra-peritoneal, intramuscular, intra-articular, subcutaneous, intranasal, intra-ocular, vaginal, rectal, or intradermal routes, depending upon the type of cancer being treated, the type of EGFR kinase inhibitor being used (for example, small molecule, antibody, RNAi, ribozyme or antisense construct), and the medical judgement of the prescribing physician as based, e.g., on the results of published clinical studies.

[0059] The amount of EGFR kinase inhibitor administered and the timing of EGFR kinase inhibitor administration will depend on the type (species, gender, age, weight, etc.) and condition of the patient being treated, the severity of the disease or condition being treated, and on the route of administration. For example, small molecule EGFR kinase inhibitors can be administered to a patient in doses ranging from 0.001 to 100 mg/kg of body weight per day or per week in single or divided doses, or by continuous infusion (see for example, International Patent Publication No. WO 01/34574). In particular, erlotinib HCl can be administered to a patient in doses ranging from 5-200 mg per day, or 100-1600 mg per week, in single or divided doses, or by continuous infusion. A preferred dose is 150 mg/day. Antibody-based EGFR kinase inhibitors, or antisense, RNAi or ribozyme constructs, can be administered to a patient in doses ranging from 0.1 to 100 mg/kg of body weight per day or per week in single or divided doses, or by continuous infusion. In some instances, dosage levels below the lower limit of the aforesaid range may be more than adequate, while in other cases still larger doses may be employed without causing any harmful side effect, provided that such larger doses are first divided into several small doses for administration throughout the day.

[0060] The EGFR kinase inhibitors and IFN α can be administered either separately or together by the same or different routes, and in a wide variety of different dosage forms. For example, the EGFR kinase inhibitor is preferably administered orally or parenterally, whereas IFN α is preferably administered parenterally. Where the EGFR kinase inhibitor is erlotinib HCl (TARCEVA®), oral administration is preferable. Both the EGFR kinase inhibitors and IFN α can be administered in single or multiple doses. In one embodiment, IFN α is administered first as a pretreatment, followed by administration of the combination of both agents (EGFR kinase inhibitor and IFN α), either separately or combined together in one formulation.

[0061] The EGFR kinase inhibitor can be administered with various pharmaceutically acceptable inert carriers in the form of tablets, capsules, lozenges, troches, hard candies, powders, sprays, creams, salves, suppositories, jellies, gels, pastes, lotions, ointments, elixirs, syrups, and the like. Administration of such dosage forms can be carried out in single or multiple doses. Carriers include solid diluents or fillers, sterile aqueous media and various non-toxic organic solvents, etc. Oral pharmaceutical compositions can be suitably sweetened and/or flavored.

[0062] The EGFR kinase inhibitor and IFN α can be combined together with various pharmaceutically acceptable

inert carriers in the form of sprays, creams, salves, suppositories, jellies, gels, pastes, lotions, ointments, and the like. Administration of such dosage forms can be carried out in single or multiple doses. Carriers include solid diluents or fillers, sterile aqueous media, and various non-toxic organic solvents, etc.

[0063] All formulations comprising proteinaceous EGFR kinase inhibitors should be selected so as to avoid denaturation and/or degradation and loss of biological activity of the inhibitor.

[0064] Methods of preparing pharmaceutical compositions comprising an EGFR kinase inhibitor are known in the art, and are described, e.g. in International Patent Publication No. WO 01/34574. Methods of preparing pharmaceutical compositions comprising IFN α are also well known in the art. In view of the teaching of the present invention, methods of preparing pharmaceutical compositions comprising both an EGFR kinase inhibitor and IFN α will be apparent from the above-cited publications and from other known references, such as Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., 18th edition (1990).

[0065] For oral administration of EGFR kinase inhibitors, tablets containing one or both of the active agents are combined with any of various excipients such as, for example, micro-crystalline cellulose, sodium citrate, calcium carbonate, dicalcium phosphate and glycine, along with various disintegrants such as starch (and preferably corn, potato or tapioca starch), alginic acid and certain complex silicates, together with granulation binders like polyvinyl pyrrolidone, sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc are often very useful for tableting purposes. Solid compositions of a similar type may also be employed as fillers in gelatin capsules; preferred materials in this connection also include lactose or milk sugar as well as high molecular weight polyethylene glycols. When aqueous suspensions and/or elixirs are desired for oral administration, the EGFR kinase inhibitor may be combined with various sweetening or flavoring agents, coloring matter or dyes, and, if so desired, emulsifying and/or suspending agents as well, together with such diluents as water, ethanol, propylene glycol, glycerin and various like combinations thereof.

[0066] For parenteral administration of either or both of the active agents, solutions in either sesame or peanut oil or in aqueous propylene glycol may be employed, as well as sterile aqueous solutions comprising the active agent or a corresponding water-soluble salt thereof. Such sterile aqueous solutions are preferably suitably buffered, and are also preferably rendered isotonic, e.g., with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal injection purposes. The oily solutions are suitable for intra-articular, intramuscular and subcutaneous injection purposes. The preparation of all these solutions under sterile conditions is readily accomplished by standard pharmaceutical techniques well known to those skilled in the art. Any parenteral formulation selected for administration of proteinaceous EGFR kinase inhibitors should be selected so as to avoid denaturation and loss of biological activity of the inhibitor.

[0067] Additionally, it is possible to topically administer either or both of the active agents, by way of, for example, creams, lotions, jellies, gels, pastes, ointments, salves and the like, in accordance with standard pharmaceutical practice. For example, a topical formulation comprising either an EGFR kinase inhibitor or IFN α in about 0.1% (w/v) to about 5% (w/v) concentration can be prepared.

[0068] For veterinary purposes, the active agents can be administered separately or together to animals using any of the forms and by any of the routes described above. In a preferred embodiment, the EGFR kinase inhibitor is administered in the form of a capsule, bolus, tablet, liquid drench, by injection or as an implant. As an alternative, the EGFR kinase inhibitor can be administered with the animal feed-stuff, and for this purpose a concentrated feed additive or premix may be prepared for a normal animal feed. The IFN α is preferably administered in the form of liquid drench, by injection or as an implant. Such formulations are prepared in a conventional manner in accordance with standard veterinary practice.

[0069] The present invention further provides a kit comprising a single container comprising both an EGFR kinase inhibitor and IFN α . The present invention further provides a kit comprising a first container comprising an EGFR kinase inhibitor and a second container comprising IFN α . In a preferred embodiment, the kit containers may further include a pharmaceutically acceptable carrier. The kit may further include a sterile diluent, which is preferably stored in a separate additional container. The kit may further include a package insert comprising printed instructions directing the use of the combined treatment as a method for treating cancer.

[0070] As used herein, the term "EGFR kinase inhibitor" refers to any EGFR kinase inhibitor that is currently known in the art or that will be identified in the future, and includes any chemical entity that, upon administration to a patient, results in inhibition of a biological activity associated with activation of the EGF receptor in the patient, including any of the downstream biological effects otherwise resulting from the binding to EGFR of its natural ligand. Such EGFR kinase inhibitors include any agent that can block EGFR activation or any of the downstream biological effects of EGFR activation that are relevant to treating cancer in a patient. Such an inhibitor can act by binding directly to the intracellular domain of the receptor and inhibiting its kinase activity. Alternatively, such an inhibitor can act by occupying the ligand binding site or a portion thereof of the EGF receptor, thereby making the receptor inaccessible to its natural ligand so that its normal biological activity is prevented or reduced. Alternatively, such an inhibitor can act by modulating the dimerization of EGFR polypeptides, or interaction of EGFR polypeptide with other proteins, or enhance ubiquitination and endocytotic degradation of EGFR. EGFR kinase inhibitors include but are not limited to low molecular weight inhibitors, antibodies or antibody fragments, antisense constructs, small inhibitory RNAs (i.e. RNA interference by dsRNA; RNAi), and ribozymes. In a preferred embodiment, the EGFR kinase inhibitor is a small organic molecule or an antibody that binds specifically to the human EGFR.

[0071] EGFR kinase inhibitors that include, for example quinazoline EGFR kinase inhibitors, pyrido-pyrimidine

EGFR kinase inhibitors, pyrimido-pyrimidine EGFR kinase inhibitors, pyrrolo-pyrimidine EGFR kinase inhibitors, pyrazolo-pyrimidine EGFR kinase inhibitors, phenylamino-pyrimidine EGFR kinase inhibitors, oxindole EGFR kinase inhibitors, indolocarbazole EGFR kinase inhibitors, phthalazine EGFR kinase inhibitors, isoflavone EGFR kinase inhibitors, quinalone EGFR kinase inhibitors, and tyrphostin EGFR kinase inhibitors, such as those described in the following patent publications, and all pharmaceutically acceptable salts and solvates of said EGFR kinase inhibitors: International Patent Publication Nos. WO 96/33980, WO 96/30347, WO 97/30034, WO 97/30044, WO 97/38994, WO 97/49688, WO 98/02434, WO 97/38983, WO 95/19774, WO 95/19970, WO 97/13771, WO 98/02437, WO 98/02438, WO 97/32881, WO 98/33798, WO 97/32880, WO 97/3288, WO 97/02266, WO 97/27199, WO 98/07726, WO 97/34895, WO 96/31510, WO 98/14449, WO 98/14450, WO 98/14451, WO 95/09847, WO 97/19065, WO 98/17662, WO 99/35146, WO 99/35132, WO 99/07701, and WO 92/20642; European Patent Application Nos. EP 520722, EP 566226, EP 787772, EP 837063, and EP 682027; U.S. Pat. Nos. 5,747,498, 5,789,427, 5,650,415, and 5,656,643; and German Patent Application No. DE 19629652. Additional non-limiting examples of low molecular weight EGFR kinase inhibitors include any of the EGFR kinase inhibitors described in Traxler, P., 1998, Exp. Opin. Ther. Patents 8(12):1599-1625.

[0072] Specific preferred examples of low molecular weight EGFR kinase inhibitors that can be used according to the present invention include [6,7-bis(2-methoxyethoxy)-4-quinazolin-4-yl]-(3-ethynylphenyl)amine (also known as OSI-774, erlotinib, or TARCEVA® (erlotinib HCl); OSI Pharmaceuticals/Genentech/Roche) (U.S. Pat. No. 5,747,498; International Patent Publication No. WO 01/34574, and Moyer, J. D. et al. (1997) Cancer Res. 57:4838-4848); CI-1033 (formerly known as PD183805; Pfizer) (Sherwood et al., 1999, Proc. Am. Assoc. Cancer Res. 40:723); PD-158780 (Pfizer); AG-1478 (University of California); CGP-59326 (Novartis); PKI-166 (Novartis); EKB-569 (Wyeth); GW-2016 (also known as GW-572016 or lapatinib ditosylate; GSK); and gefitinib (also known as ZD1839 or IRESSA™; AstraZeneca) (Woodburn et al., 1997, Proc. Am. Assoc. Cancer Res. 38:633). A particularly preferred low molecular weight EGFR kinase inhibitor that can be used according to the present invention is [6,7-bis(2-methoxyethoxy)-4-quinazolin-4-yl]-(3-ethynylphenyl)amine (i.e. erlotinib), its hydrochloride salt (i.e. erlotinib HCl, TARCEVA®), or other salt forms (e.g. erlotinib mesylate).

[0073] Antibody-based EGFR kinase inhibitors include any anti-EGFR antibody or antibody fragment that can partially or completely block EGFR activation by its natural ligand. Non-limiting examples of antibody-based EGFR kinase inhibitors include those described in Modjtahedi, H., et al., 1993, Br. J. Cancer 67:247-253; Teramoto, T., et al., 1996, Cancer Res. 56:639-645; Goldstein et al., 1995, Clin. Cancer Res. 1:1311-1318; Huang, S. M., et al., 1999, Cancer Res. 59:1236-1243. Thus, the EGFR kinase inhibitor can be the monoclonal antibody Mab E7.6.3 (Yang, X. D. et al. (1999) Cancer Res. 59:1236-43), or Mab C225 (ATCC Accession No. HB-8508), or an antibody or antibody fragment having the binding specificity thereof. Suitable monoclonal antibody EGFR kinase inhibitors include, but are not limited to, IMC-C225 (also known as cetuximab or

ERBITUX™; Imclone Systems), ABX-EGF (Abgenix), EMD 72000 (Merck KgaA, Darmstadt), RH3 (York Medical Bioscience Inc.), and MDX-447 (Medarex/Merck KgaA).

[0074] Additional antibody-based EGFR kinase inhibitors can be raised according to known methods by administering the appropriate antigen or epitope to a host animal selected, e.g., from pigs, cows, horses, rabbits, goats, sheep, and mice, among others. Various adjuvants known in the art can be used to enhance antibody production.

[0075] Although antibodies useful in practicing the invention can be polyclonal, monoclonal antibodies are preferred. Monoclonal antibodies against EGFR can be prepared and isolated using any technique that provides for the production of antibody molecules by continuous cell lines in culture. Techniques for production and isolation include but are not limited to the hybridoma technique originally described by Kohler and Milstein (Nature, 1975, 256: 495-497); the human B-cell hybridoma technique (Kosbor et al., 1983, Immunology Today 4:72; Cote et al., 1983, Proc. Natl. Acad. Sci. USA 80: 2026-2030); and the EBV-hybridoma technique (Cole et al., 1985, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

[0076] Alternatively, techniques described for the production of single chain antibodies (see, e.g., U.S. Pat. No. 4,946,778) can be adapted to produce anti-EGFR single chain antibodies. Antibody-based EGFR kinase inhibitors useful in practicing the present invention also include anti-EGFR antibody fragments including but not limited to F(ab')₂ fragments, which can be generated by pepsin digestion of an intact antibody molecule, and Fab fragments, which can be generated by reducing the disulfide bridges of the F(ab')₂ fragments. Alternatively, Fab and/or scFv expression libraries can be constructed (see, e.g., Huse et al., 1989, Science 246: 1275-1281) to allow rapid identification of fragments having the desired specificity to EGFR.

[0077] Techniques for the production and isolation of monoclonal antibodies and antibody fragments are well-known in the art, and are described in Harlow and Lane, 1988, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, and in J. W. Goding, 1986, Monoclonal Antibodies: Principles and Practice, Academic Press, London. Humanized anti-EGFR antibodies and antibody fragments can also be prepared according to known techniques such as those described in Vaughn, T. J. et al., 1998, Nature Biotech. 16:535-539 and references cited therein, and such antibodies or fragments thereof are also useful in practicing the present invention.

[0078] EGFR kinase inhibitors for use in the present invention can alternatively be based on antisense oligonucleotide constructs. Anti-sense oligonucleotides, including anti-sense RNA molecules and anti-sense DNA molecules, would act to directly block the translation of EGFR mRNA by binding thereto and thus preventing protein translation or increasing mRNA degradation, thus decreasing the level of EGFR kinase protein, and thus activity, in a cell. For example, antisense oligonucleotides of at least about 15 bases and complementary to unique regions of the mRNA transcript sequence encoding EGFR can be synthesized, e.g., by conventional phosphodiester techniques and administered by e.g., intravenous injection or infusion. Methods for using antisense techniques for specifically inhibiting gene expression of genes whose sequence is known are well

known in the art (e.g. see U.S. Pat. Nos. 6,566,135; 6,566,131; 6,365,354; 6,410,323; 6,107,091; 6,046,321; and 5,981,732).

[0079] Small inhibitory RNAs (siRNAs) can also function as EGFR kinase inhibitors for use in the present invention. EGFR gene expression can be reduced by contacting the tumor, subject or cell with a small double stranded RNA (dsRNA), or a vector or construct causing the production of a small double stranded RNA, such that expression of EGFR is specifically inhibited (i.e. RNA interference or RNAi). Methods for selecting an appropriate dsRNA or dsRNA-encoding vector are well known in the art for genes whose sequence is known (e.g. see Tuschl, T., et al. (1999) *Genes Dev.* 13(24):3191-3197; Elbashir, S. M. et al. (2001) *Nature* 411:494-498; Hannon, G. J. (2002) *Nature* 418:244-251; McManus, M. T. and Sharp, P. A. (2002) *Nature Reviews Genetics* 3:737-747; Bremmelkamp, T. R. et al. (2002) *Science* 296:550-553; U.S. Pat. Nos. 6,573,099 and 6,506,559; and International Patent Publication Nos. WO 01/36646, WO 99/32619, and WO 01/68836).

[0080] Ribozymes can also function as EGFR kinase inhibitors for use in the present invention. Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Engineered hairpin or hammerhead motif ribozyme molecules that specifically and efficiently catalyze endonucleolytic cleavage of EGFR mRNA sequences are thereby useful within the scope of the present invention. Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, which typically include the following sequences, GUA, GuU, and GUC. Once identified, short RNA sequences of between about 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site can be evaluated for predicted structural features, such as secondary structure, that can render the oligonucleotide sequence unsuitable. The suitability of candidate targets can also be evaluated by testing their accessibility to hybridization with complementary oligonucleotides, using, e.g., ribonuclease protection assays.

[0081] Both antisense oligonucleotides and ribozymes useful as EGFR kinase inhibitors can be prepared by known methods. These include techniques for chemical synthesis such as, e.g., by solid phase phosphoramidite chemical synthesis. Alternatively, anti-sense RNA molecules can be generated by in vitro or in vivo transcription of DNA sequences encoding the RNA molecule. Such DNA sequences can be incorporated into a wide variety of vectors that incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Various modifications to the oligonucleotides of the invention can be introduced as a means of increasing intracellular stability and half-life. Possible modifications include but are not limited to the addition of flanking sequences of ribonucleotides or deoxyribonucleotides to the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2'-O-methyl rather than phosphodiesterase linkages within the oligonucleotide backbone.

[0082] The invention also encompasses a pharmaceutical composition that is comprised of an EGFR kinase inhibitor and IFN α combination in combination with a pharmaceutically acceptable carrier.

[0083] Preferably the composition is comprised of a pharmaceutically acceptable carrier and a non-toxic therapeutically effective amount of an EGFR kinase inhibitor compound and IFN α combination (including pharmaceutically acceptable salts of each component thereof).

[0084] Moreover, within this preferred embodiment, the invention encompasses a pharmaceutical composition for the treatment of disease, the use of which results in the inhibition of growth of neoplastic cells, benign or malignant tumors, or metastases, comprising a pharmaceutically acceptable carrier and a non-toxic therapeutically effective amount of an EGFR kinase inhibitor compound and IFN α combination (including pharmaceutically acceptable salts of each component thereof).

[0085] The term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases or acids. When a compound of the present invention is acidic, its corresponding salt can be conveniently prepared from pharmaceutically acceptable non-toxic bases, including inorganic bases and organic bases. Salts derived from such inorganic bases include aluminum, ammonium, calcium, copper (cupric and cuprous), ferric, ferrous, lithium, magnesium, manganese (manganic and manganous), potassium, sodium, zinc and the like salts. Particularly preferred are the ammonium, calcium, magnesium, potassium and sodium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, as well as cyclic amines and substituted amines such as naturally occurring and synthesized substituted amines. Other pharmaceutically acceptable organic non-toxic bases from which salts can be formed include ion exchange resins such as, for example, arginine, betaine, caffeine, choline, N,N'-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine and the like.

[0086] When a compound of the present invention is basic, its corresponding salt can be conveniently prepared from pharmaceutically acceptable non-toxic acids, including inorganic and organic acids. Such acids include, for example, acetic, benzenesulfonic, benzoic, camphorsulfonic, citric, ethanesulfonic, fumaric, gluconic, glutamic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, pantoic, pantothenic, phosphoric, succinic, sulfuric, tartaric, p-toluenesulfonic acid and the like. Particularly preferred are citric, hydrobromic, hydrochloric, maleic, phosphoric, sulfuric and tartaric acids.

[0087] The pharmaceutical compositions of the present invention comprise an EGFR kinase inhibitor compound and IFN α combination (including pharmaceutically acceptable salts of each component thereof) as active ingredient, a pharmaceutically acceptable carrier and optionally other

therapeutic ingredients or adjuvants. Other therapeutic agents may include those cytotoxic, chemotherapeutic or anti-cancer agents, or agents which enhance the effects of such agents, as listed above. The compositions include compositions suitable for oral, rectal, topical, and parenteral (including subcutaneous, intramuscular, and intravenous) administration, although the most suitable route in any given case will depend on the particular host, and nature and severity of the conditions for which the active ingredient is being administered. The pharmaceutical compositions may be conveniently presented in unit dosage form and prepared by any of the methods well known in the art of pharmacy.

[0088] In practice, the compounds represented by an EGFR kinase inhibitor compound and IFN α combination (including pharmaceutically acceptable salts of each component thereof) of this invention can be combined as the active ingredient in intimate admixture with a pharmaceutical carrier according to conventional pharmaceutical compounding techniques. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g. oral or parenteral (including intravenous). Thus, the pharmaceutical compositions of the present invention can be presented as discrete units suitable for oral administration such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient. Further, the compositions can be presented as a powder, as granules, as a solution, as a suspension in an aqueous liquid, as a non-aqueous liquid, as an oil-in-water emulsion, or as a water-in-oil liquid emulsion. In addition to the common dosage forms set out above, an EGFR kinase inhibitor compound and IFN α combination (including pharmaceutically acceptable salts of each component thereof) may also be administered by controlled release means and/or delivery devices. The combination compositions may be prepared by any of the methods of pharmacy. In general, such methods include a step of bringing into association the active ingredients with the carrier that constitutes one or more necessary ingredients. In general, the compositions are prepared by uniformly and intimately admixing the active ingredient with liquid carriers or finely divided solid carriers or both. The product can then be conveniently shaped into the desired presentation.

[0089] Thus, the pharmaceutical compositions of this invention may include a pharmaceutically acceptable carrier and an EGFR kinase inhibitor compound and IFN α combination (including pharmaceutically acceptable salts of each component thereof). An EGFR kinase inhibitor compound and IFN α combination (including pharmaceutically acceptable salts of each component thereof), can also be included in pharmaceutical compositions in combination with one or more other therapeutically active compounds. Other therapeutically active compounds may include those cytotoxic, chemotherapeutic or anti-cancer agents, or agents which enhance the effects of such agents, as listed above.

[0090] Thus in one embodiment of this invention, a pharmaceutical composition can comprise an EGFR kinase inhibitor compound and IFN α in combination with an anticancer agent, wherein said anti-cancer agent is a member selected from the group consisting of alkylating drugs, antimetabolites, microtubule inhibitors, podophyllotoxins, antibiotics, nitrosoureas, hormone therapies, kinase inhibitors, activators of tumor cell apoptosis, and antiangiogenic agents.

[0091] The pharmaceutical carrier employed can be, for example, a solid, liquid, or gas. Examples of solid carriers include lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, and stearic acid. Examples of liquid carriers are sugar syrup, peanut oil, olive oil, and water. Examples of gaseous carriers include carbon dioxide and nitrogen.

[0092] In preparing the compositions for oral dosage form, any convenient pharmaceutical media may be employed. For example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, and the like may be used to form oral liquid preparations such as suspensions, elixirs and solutions; while carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents, and the like may be used to form oral solid preparations such as powders, capsules and tablets. Because of their ease of administration, tablets and capsules are the preferred oral dosage units whereby solid pharmaceutical carriers are employed. Optionally, tablets may be coated by standard aqueous or nonaqueous techniques.

[0093] A tablet containing the composition of this invention may be prepared by compression or molding, optionally with one or more accessory ingredients or adjuvants. Compressed tablets may be prepared by compressing, in a suitable machine, the active ingredient in a free-flowing form such as powder or granules, optionally mixed with a binder, lubricant, inert diluent, surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine, a mixture of the powdered compound moistened with an inert liquid diluent. Each tablet preferably contains from about 0.05 mg to about 5 g of the active ingredient and each cachet or capsule preferably contains from about 0.05 mg to about 5 g of the active ingredient.

[0094] For example, a formulation intended for the oral administration to humans may contain from about 0.5 mg to about 5 g of active agent, compounded with an appropriate and convenient amount of carrier material that may vary from about 5 to about 95 percent of the total composition. Unit dosage forms will generally contain between from about 1 mg to about 2 g of the active ingredient, typically 25 mg, 50 mg, 100 mg, 200 mg, 300 mg, 400 mg, 500 mg, 600 mg, 800 mg, or 1000 mg.

[0095] Pharmaceutical compositions of the present invention suitable for parenteral administration may be prepared as solutions or suspensions of the active compounds in water. A suitable surfactant can be included such as, for example, hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof in oils. Further, a preservative can be included to prevent the detrimental growth of microorganisms.

[0096] Pharmaceutical compositions of the present invention suitable for injectable use include sterile aqueous solutions or dispersions. Furthermore, the compositions can be in the form of sterile powders for the extemporaneous preparation of such sterile injectable solutions or dispersions. In all cases, the final injectable form must be sterile and must be effectively fluid for easy syringability. The pharmaceutical compositions must be stable under the conditions of manufacture and storage; thus, preferably should be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a

solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol and liquid polyethylene glycol), vegetable oils, and suitable mixtures thereof.

[0097] Pharmaceutical compositions of the present invention can be in a form suitable for topical use such as, for example, an aerosol, cream, ointment, lotion, dusting powder, or the like. Further, the compositions can be in a form suitable for use in transdermal devices. These formulations may be prepared, utilizing an EGFR kinase inhibitor compound and IFN α combination (including pharmaceutically acceptable salts of each component thereof) of this invention, via conventional processing methods. As an example, a cream or ointment is prepared by admixing hydrophilic material and water, together with about 5 wt % to about 10 wt % of the compound, to produce a cream or ointment having a desired consistency.

[0098] Pharmaceutical compositions of this invention can be in a form suitable for rectal administration wherein the carrier is a solid. It is preferable that the mixture forms unit dose suppositories. Suitable carriers include cocoa butter and other materials commonly used in the art. The suppositories may be conveniently formed by first admixing the composition with the softened or melted carrier(s) followed by chilling and shaping in molds.

[0099] In addition to the aforementioned carrier ingredients, the pharmaceutical formulations described above may include, as appropriate, one or more additional carrier ingredients such as diluents, buffers, flavoring agents, binders, surface-active agents, thickeners, lubricants, preservatives (including anti-oxidants) and the like. Furthermore, other adjuvants can be included to render the formulation isotonic with the blood of the intended recipient. Compositions containing an EGFR kinase inhibitor compound and IFN α combination (including pharmaceutically acceptable salts of each component thereof) may also be prepared in powder or liquid concentrate form.

[0100] Dosage levels for the compounds of the combination of this invention will be approximately as described herein, or as described in the art for these compounds. It is understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

[0101] This invention will be better understood from the Experimental Details that follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter, and are not to be considered in any way limited thereto.

[0102] Experimental Details:

[0103] Introduction

[0104] The presence of and signaling through growth factor receptors on tumor cells has implications for receptor-specific cancer therapy. For the estrogen receptor, up to 50% of breast cancer patients have receptor negative tumors and are ineligible for therapy based on binding to this ligand (Elledge R M, Fuqua S A W. Estrogen and progesterone

receptors in: Harris J R, et al., eds. Diseases of the breast 2nd Ed, (2000) Philadelphia: Lippincott, Williams and Wilkins, p 471). Similarly the applicability of a highly active and novel therapeutic inhibitor, Herceptin (now clinically available) is limited as only 20% of breast cancers express its receptor, HER-2/neu (encoded by c-erbB-2 oncogene) (Paik, S., E. R., et al. (1990) J. Clin. Oncol.; 8: 103-112). A similar problem may develop with the use of agents such as erlotinib (OSI-774, with a trade name of 'TARCEVA®') that targets the HER1/EGFR via blockade of tyrosine kinase activity and is well advanced in clinical trials. In NSW, colon cancers are the most common cancers of internal organs and there are no curative therapies for relapsed or inoperable patients (Supramanian R, et al. (1998) Survival from Cancer in New South Wales in 1980 to 1995, Sydney, NSW Cancer Council). New therapeutic strategies are needed for progressive tumors. HER1/EGFR and its signals contribute to many cellular processes including cell cycle progression, angiogenesis, metastatic cancer spread, and inhibition of apoptosis (Wells, A., (1999) Int. J. Biochem. Cell. Biol. 31:637-643; Perry, J. E., et al. (1998) Prostate 35:117-124; Woodburn, J. R., (1999) Pharmacol. Ther. 82:241-250). In colon cancer, HER1/EGFR expression is dysregulated possibly playing a role in the proliferation and progression of the tumors (Grunwald, V., and Hidalgo, (2003) M. J., Natl. Cancer Inst. 95:851-867). Increased cell replication can occur through enhanced growth stimulation by external ligand or by mutation and autocrine growth enhancement in the absence of external stimulation (Ge, H., et al. (2002) Int. J. Cancer 98:357-361). High levels or over-expression of HER1/EGFR are thus associated with aggressive biological behavior and poor clinical outcome (Grandis J R, et al. (1998) J. Natl. Cancer Inst. 90:824-832). Activity of agents that target HER1/EGFR may depend on the numbers of HER1/EGFR expressed (Bos, M., et al. (1997) Clin. Cancer Res. 3:2099-2106). An in vivo study has shown that anti-HER1/EGFR efficacy depended on HER1/EGFR concentration rather than on tumor histology (Hambek, M. H., et al. (2001) Cancer Res. 61:1045-1049). Recent clinical research suggests that effective inhibition may not only be related to the expression of HER1/EGFR, but also to its activation status (Grunwald, V., and Hidalgo, M. J. (2003) Natl. Cancer Inst. 95:851-867). Since only few patients benefit from such therapy (whether as a single agent or combined with chemotherapy) (Grunwald, V., and Hidalgo, (2003) M. J., Natl. Cancer Inst. 95:851-867), enhancing inhibition or the proportion of susceptible tumors may be clinically relevant. One approach may be to enhance targeting of the HER1/EGFR. We have shown that IFN α -induced growth inhibition of some human colon cancer cell lines is paralleled by up-regulation of HER1/EGFR and this does not inhibit the EGF-HER1/EGFR pathway, as the antiproliferative effect of IFN α was reduced by adding EGF (Yang, J-L., et al. (2004) Gut 53:123-129). This is also the case in primary human epidermoid, cervical and endometrial tumor cells (Budillon, A, et al. (1991) Cancer Res. 51:1294-1299; Heise, H., et al. (1995) Anti-Cancer Drugs 6:686-692; Scambia, G, et al. (1994) Int. J. Cancer 58:769-773). Regardless of the mechanism, the up-regulation of HER1/EGFR in the presence of IFN α , provided that HER1/EGFR signaling is maintained, may identify cells that may be susceptible to enhanced growth inhibition by HER1/EGFR blockers. We hypothesized that IFN- α might improve the efficacy of HER1/EGFR-targeted treatment. This study aimed to determine

whether combining erlotinib (a specific HER1/EGFR inhibitor) with IFN- α increases the anti-tumor effect of either alone.

[0105] Materials and Methods

[0106] Drugs.

[0107] IFN- α was a kind gift from Roche Pharmaceuticals, Dee Why, Sydney, Australia. The selective HER1/EGFR inhibitor, erlotinib, was kindly provided by OSI Pharmaceuticals, Uniondale, N.Y., USA, as the hydrochloride salt, erlotinib HCl (TARCEVA®).

[0108] Cell Lines.

[0109] Human colon cancer cell lines, DLD-1, HCT116, HT29, KM12SM, LOVO, SW480 and SW620 were purchased from the American Type of Cell Culture. LIM2408 and LIM2099 were obtained from Dr Robert Whitehead, The Ludwig Institute for Cancer Research in Melbourne.

[0110] Cell Culture.

[0111] Cells were grown in minimum essential medium (MEM: GIBCO, Grand Island, N.Y., USA), supplemented with 10% heat-inactivated fetal bovine serum (FCS), 2 mM L-glutamine, penicillin and streptomycin at 37° C. in a humidified 5% CO₂ and 95% atmosphere. Cells were fed every 3 to 4 days, and harvested by brief incubation in 0.02% EDTA-PBS (ICN, Aurora, Ohio, USA).

[0112] Crystal Violet Colorimetric Assay:

[0113] Cells were cultured in 24 well plates at 5×10^4 per well, pretreated with 100 IU/ml of IFN α or DMSO for 72 h, then removed the medium and washed with PBS. Cells were then treated with different agents for the required time period, then washed with DPBS (phosphate-buffered saline with divalent cations: 1 mM CaCl₂ and 0.5 mM MgCl₂, SIGMA, St. Louis, Mo., USA) and stained with 0.5% crystal violet, rinsed in distilled water until all excess stain had been removed, and air dried. One mL/well elution solution [1:1 0.1M Na⁺ citrate (pH4.2): 100% ethanol was added and mixed gently for 30 min before transferring 200 μ L of the solution from each well into a 96-well plate. Light absorbance of the solution was measured at 540 nm on the plate reader (TECAN, Grodig, Salzburg, Austria). The growth of experimental and control cells was compared. Duplicate experiments with triplicate samples were performed for all cell lines.

[0114] Immunofluorescent Flow Cytometry.

[0115] The cell-surface HER1/EGFR levels at 72 h and 96 h of culture, with or without IFN- α (50 or 100 IU/ml), were measured by immunofluorescent flow cytometry. Cells were seeded in 60 $\times$ 15 mm dishes (FALCON™, BD) and treated with 100 IU/mL of IFN- α . The treated and untreated cells were harvested at different time intervals using 0.02% EDTA in PBS, then washed twice with ice-cold PBS and counted. The mouse monoclonal antibody against the extracellular domain of human HER1/EGFR (Catlog No: MO886, DAKO) diluted 1 in 100 in PBS was added to the cells (1×10^6 cells in 100 μ L volume), while an irrelevant IgG 2b was used as the isotype-matched antibody control. After 60 min on ice, cells were washed and incubated with 100 μ L of fluorescein-isothiocyanate (FITC)-conjugated goat-anti-mouse IgG (Catlog No: 81-6511, ZYMED), 1 in 200 dilution in PBS, on ice for 30 min in the dark. The cells were

then washed again twice and resuspended in 1% formaldehyde in PBS. Cell surface immunofluorescence was analysed on a FACSCalibur (BD) using CELLQuest software equipped with a 5 W argon ion laser tuned to 488 nm at 200 mW. Levels of protein expression were estimated as the geometric mean of fluorescence intensity (MFI) of anti-HER1/EGFR minus that of the isotype-matched antibody control. Ten thousand singlet and viable cells of the individual treated and control samples were measured. Duplicate experiments with triplicate samples were performed for all cells.

[0116] Definition and classification of the effect of combined drug treatment.

[0117] Two methods were used to evaluate the effect of combination treatment with erlotinib and IFN α .

[0118] (1) Classification Index: The potentiation was estimated by multiplying the percent of cells remaining (% of growth) compared to control of each individual agent. The classification index was calculated as previously described (Goldstein D., et al. (1989) Cancer Res. 49:2698-2702) with mild modification:

[0119] Supra-additivity was defined as: % AB/(% A $\times$ % B)>0.9

[0120] Additivity was defined as: % AB/(% A $\times$ % B)=0.9-1

[0121] And subadditivity was defined as: % AB/(% A $\times$ % B)<1 (Where A and B are the effects of each individual agent and AB is the effect of the combination).

[0122] (2) Arithmetic and statistical assay: Additivity was concluded if AB (% of inhibition) surpassed A alone plus B alone. Supra-additivity was concluded if the difference of enhanced efficacy in AB versus A alone plus B alone was statistically significant. (Where A and B are the effects of each individual agent and AB is the effect of the combination).

[0123] Statistical analysis: Data were presented as mean plus or minus standard deviation of the mean of all replicates, or mean plus or minus variation of the mean from all repeated experiments. The non-parametric Kruskal-Wallis method and a post-hoc Bonferroni or Scheffe test were used to detect percent cell growth across/between cell lines. The paired Student's t-test was used to evaluate the significance of cell-surface HER1/EGFR levels before and after treatment by IFN α . Statistical values of p(2-tail)<0.05 or 95% confidence interval excluding zero were considered significant. Statistical analysis was performed using the SPSS/Win11.5 software (SPSS, Inc, Chicago, Ill.).

[0124] Results

[0125] Cell-surface HER1/EGFR expression by untreated cell lines: All cell lines were measured first to obtain an untreated level of HER1/EGFR. Cells were harvested after 72 h of culture and measured by flow cytometry. The HER1/EGFR value was calculated as the geometric mean fluorescence intensity of anti-HER1/EGFR detection minus that of isotype control. SW620 cells were HER1/EGFR-negative. Other cell lines including DLD-1, HCT116, HT29, KM12SM, LIM2099, LIM2408, LOVO and SW480 all positively expressed HER1/EGFR.

[0126] Association between IFN α treatment and HER1/EGFR expression in colon cancer cell lines.

[0127] The relationship between HER1/EGFR expression and IFN α treatment was assessed in all cell lines. Cells were all cultured with or without 100 IU/mL of IFN α (which mimics serum levels in humans given subcutaneous interferon (Robinson S. P., et al. (1990) *Breast Cancer Res. Treat.*; 5:95-101) for 72 and 96 h. The cell-surface HER1/EGFR expression at 72 h of culture from untreated and treated groups of individual cell lines detected using anti-HER1/EGFR antibody minus isotype control is shown in **FIG. 1**. IFN α treatment was associated with significant up-regulation of cell-surface HER1/EGFR expression in 7 of 8 HER1/EGFR-positive cell lines, including DLD-1 (HER1/EGFR level (mean plus/minus standard deviation of the mean) treated versus that untreated: 17.89+0.60/10.86+0.51, paired t test, $p=0.005$; 95% confidence interval of the difference: 4.88–9.16), HCT116 (22.75+0.79/7.80+0.22, $p<0.001$; 13.52–16.36), HT29 (11.12+0.20/8.58+0.24, $p=0.004$; 1.82–3.27), LIM2099 (22.19+0.56/12.82+0.52, $p=0.001$; 7.87–10.86), LIM2408 (22.93+0.53/10.77+0.21, $p=0.001$; 10.35–13.97), LOVO (14.77+0.34/8.12+0.25, $p=0.003$; 5.2–8.1), and SW480 (27.09+0.76/14.10+0.33, $p=0.002$; 10.66–15.32) cells. In contrast, HER1/EGFR expression levels were not increased in KM12SM cells after IFN α treatment. In addition, IFN α treatment was unable to induce HER1/EGFR expression by the HER1/EGFR-negative SW620 cells. Consistently, at 96 h of culture the cell-surface HER1/EGFR expression from untreated and treated groups of individual cell lines followed the same trend. Since up-regulation of HER1/EGFR expression was sustained between 72 and 96 h, we thus chose 72 h for IFN α pretreatment and 96 h for combination treatment introduced below.

[0128] Anti-proliferative effect of different doses of erlotinib and IFN α on LIM2408 cells.

[0129] We compared a combination of different doses of erlotinib (0.1–10 μ g/ml) and IFN α (50 and 100 IU/mL), with IFN α or erlotinib alone in vitro on LIM2408 cells. The results are shown in both **FIG. 2a** for 50 IU/mL of IFN α combined with different doses of erlotinib and **FIG. 2b** for 100 IU/mL of IFN α with erlotinib. For the 50 IU/mL IFN α experiment (**FIG. 2a**), the nonparametric Kruskal-Wallis test was used to determine if there was a significant difference in percent cell growth across all individual groups at all of different erlotinib concentration points, including an extra group adding values of 'IFN α 50 IU/mL alone' plus 'erlotinib alone' together. A significant difference ($H=30.429$, $p<0.001$) was noticed. We then compared the extra group with each of the other individual groups by a post-hoc Bonferroni multiple comparisons test to detect difference between any two selected groups. Significant favourable anti-proliferative effects were observed in two groups. They were (1) the group of IFN α 50 IU/mL plus erlotinib ($p<0.001$; mean percent inhibition difference: 28.69; 95% confidence interval: 9.42–35.96) and (2) the group of 100 IU/mL of IFN α pretreatment (then washing) followed by IFN α 50 IU/mL plus erlotinib ($p<0.001$; 39.18; 25.91–52.45). Consistently, there was a significant difference in percentage of cell growth across all individual groups (Kruskal-Wallis, $H=30.841$, $p<0.001$) in the 100 IU/mL IFN α experiment (**FIG. 2b**). Compared with the extra group of 'IFN α 100 IU/mL alone' plus 'erlotinib alone', positive

mean percent inhibition difference was observed in both the group of IFN α 100 IU/mL plus erlotinib (11.39) and the group of 100 IU/mL of IFN α pretreatment (then washing) followed by IFN α 1000 IU/mL plus erlotinib (28.57). However, only the group with IFN α pretreatment had significant antiproliferative benefit (Bonferroni, $p<0.001$; 95% CI: 14.91–42.23). Based on the experiments on the doses of both drugs mentioned above, the optimal doses for combining IFN- α (50 IU/mL) with erlotinib (2 μ g/ml) were selected for further experiments to avoid an eclipsic effect.

[0130] Anti-proliferative effect of combination of erlotinib with IFN α on different colon cancer cell lines.

[0131] We finally determined whether a combination of IFN- α and erlotinib has an additive or supra-additive effect on cell lines with up-regulated HER1/EGFR. In addition, we also tested whether initial IFN α treatment (pre-incubation with IFN α to maximize the modulation of HER1/EGFR) might improve the effects of combined treatment. All cell lines including HER1/EGFR negative or down-regulated cells were treated with (1) Vehicle, (2) IFN- α alone (50 IU/mL), (3) erlotinib alone (2 μ g/mL), (4) IFN- α (50 IU/mL) plus erlotinib (2 μ g/mL), (5) IFN- α (100 IU/mL) pre-treatment for 72 h (then washing) followed by erlotinib (2 μ g/mL), and (6) IFN- α (100 IU/mL) pre-treatment for 72 h (then washing) followed by IFN- α (50 IU/mL) plus erlotinib (2 μ g/ml). After 96 h, we assessed cell proliferation in all groups by crystal violet colorimetric assay and evaluated the drugs' effects by the two methods.

[0132] Concurrent treatment with IFN α and erlotinib showed an additivity or supra-additivity (up to 25.07% supra-addition) in 6 cell lines (3 supra-additive and 3 additive) by arithmetic and statistical assay (**FIG. 4**) or in 7 cell lines (4 supra-additive and 3 additive) by classification index method (**FIG. 5**). These cell lines were all HER1/EGFR-upregulated. IFN- α pre-treatment followed by combined IFN- α and erlotinib had a supra-additive effect (up to 42.74% supra-addition) in all seven cell lines in which IFN- α upregulated HER1/EGFR expression (**FIGS. 4 and 5**; **FIG. 3**). This was substantially enhanced compared to only concurrent treatment.

[0133] Discussion

[0134] In this study we showed that combined IFN- α and erlotinib, a specific HER1/EGFR inhibitor can have an additive or supra-additive inhibitory effect on the growth of human colon cancer cells detected by crystal violet colorimetric assay and evaluated by both the classification index and the arithmetic and statistical assay, suggesting that the two drugs are not antagonistic and may be synergistic in selected colon cancer cell lines in which IFN α treatment is associated with up-regulation of HER1/EGFR expression. This may have clinical implications for identifying patients and improving treatment that inhibits HER1/EGFR.

[0135] HER1/EGFR is a member of a subfamily of four closely related receptors: HER1/EGFR (or ErbB1), HER2 (ErbB2), HER3 (ErbB3), and HER4 (ErbB4) (Wells, A., (1999) *Int. J. Biochem. Cell. Biol.* 31:637-643). Ligand activation of these receptors, which exist as inactive monomers in epithelial, mesenchymal, and neuronal tissues, causes homo- or hetero-dimerization between HER1/EGFR and another member of the erb receptor family. The tyrosine kinase intracellular domain of the receptor is then activated,

with autophosphorylation of the intracellular domain, initiating a cascade of intracellular events (Wells, A., (1999) *Int. J. Biochem. Cell. Biol.* 31:637-643). The down-stream signaling pathway involves activation of ras-MAPK (MEK-ERK) (mitogen-activated protein/extracellular signal-regulated kinase), PI3K (phosphatidylinositol 3'-kinase)-Akt and phospholipase-C-gamma-1 (PLC γ), which in turn, activates several nuclear proteins, and contributes to many cellular processes including cell cycle progression, angiogenesis, metastatic cancer spread, and inhibition of apoptosis (Wells, A., (1999) *Int. J. Biochem. Cell. Biol.* 31:637-643; Perry, J. E., et al. (1998) *Prostate* 35:117-124; Woodburn, J. R., (1999) *Pharmacol. Ther.* 82:241-250). erlotinib is a small molecule and binds competitively to the ATP binding pocket of the HER1/EGFR tyrosine kinases, thus inhibiting phosphorylation of the receptor (Grunwald, V., and Hidalgo, (2003) *M. J., Natl. Cancer Inst.* 95:851-867). IFN α binds to cell membrane receptors, IFN α R1 and IFN α R2 (Chill, J. H., et al. (2002) *Biochem.* 41:3575-3585), leading to activation of IFN α associated Janus kinases (JAK), Jak1 and Tyk2. These are nonreceptor tyrosine kinases that mediate cytokine-induced signal transduction (Leaman, D. W., et al. (1996) *Mol. Cell. Biol.* 16:369-375). The activated JAKs phosphorylate and activate cytoplasmic signalling proteins including STAT (signalling transducers and activators of transcription) transcription factors. IFN α , via the JAK-STAT pathway, regulates target gene expression, leading to cell growth inhibition (Rane, S. G. and Reddy, E. P. (2000) *Oncogene* 19:5662-5679).

[0136] The supra-additive anti-proliferative effect achieved by combining erlotinib with IFN α may indicate that either the process of HER1/EGFR upregulation itself has the potential to enhance sensitivity to its inhibitors, or may be a manifestation of a more fundamental downstream effect of IFN α that may enhance the effectiveness of HER1/EGFR inhibitors. The evidence that concurrent IFN- α enhanced the effect of erlotinib on tumor growth and pre-treatment with IFN- α for 72 h enhanced that growth inhibition in HER1/EGFR up-regulated colon cancer cell lines, but not in HER1/EGFR-negative or insensitive cell lines supports the notion that IFN α induced upregulation of HER1/EGFR expression may play an important role in the mechanism of enhanced efficacy of the two drugs. HER1/EGFR may be upregulated through either a direct effect of IFN- α on HER1/EGFR synthesis and function (since IFN- α induces expression of IFN regulatory factor-1, an important transcription factor that modulates expression of many genes including HER1/EGFR) or a secondary effect via changes in cell growth status (reviewed in Yang, J.-L., et al. (2004) *Gut* 53:123-129). Thus, increased receptor expression could be part of a homeostatic cellular response to strong anti-proliferative stimuli. Alternatively, since erlotinib inhibits activation of the HER1/EGFR intracellular tyrosine kinase and down-stream signaling pathways such as PI3/Akt and Ras/Raf/MAPK a downstream interaction may enhance inhibition of cell proliferation (Grunwald, V., and Hidalgo, (2003) *M. J., Natl. Cancer Inst.* 95:851-867). The anti-proliferative effects of both IFN α and HER1/EGFR inhibitors are ultimately mediated via increased apoptosis using a caspase pathway (Panaretakis, T., et al. (2003) *Oncogene* 22:4543-4556).

[0137] Regardless of the mechanism, the IFN α -induced upregulation of HER1/EGFR may indicate that HER1/EGFR signaling can be modulated and it may be an alter-

native to positive expression of the receptor as a predictor for identifying tumor cells/patients that may be susceptible to enhanced growth inhibition by combining HER1/EGFR blockers with interferon.

[0138] In the present study, combination treatment with erlotinib and IFN α caused decreased anti-proliferative effects in the one HER1/EGFR positive cell line, KM12SM in which there was no IFN α induced upregulation of HER1/EGFR. As expected there was no alteration in antiproliferative effects in the receptor-negative cell line, SW620 (FIG. 4). This suggests that the mechanism of action of combining erlotinib with IFN α is complex and testing only the baseline level of HER1/EGFR expression is poorly predictive of therapeutic effects targeting the receptor. Resistance to HER1/EGFR inhibitors and/or IFN α treatment may reflect a dysregulation of the second messenger pathway downstream from their receptors. For an example, loss of PTEN in HER1/EGFR expressing tumor cells counteracts the antitumor action of HER1/EGFR inhibitors (She, Q. B., et al. (2003) *Clin. Cancer Res.* 9:4340-4346; Bianco, R., et al. (2003) *Oncogene* 22:2812-2822); this can be overcome by restoring PTEN function or by pharmacologic modulation of constitutive PI3K/Akt pathway signaling (Bianco, R., et al. (2003) *Oncogene* 22:2812-2822). Studies of potential common pathways and their regulators may identify the source of synergy and ways to further enhance the effect.

[0139] Effect of Erlotinib on Bladder Cancer Cell Lines, and Enhancement Sensitivity by Combination with IFN- α .

[0140] It is known that 40-60% of bladder cancers over-express EGFR which provides an opportunity for targeted therapy. The aims of this study were: (1) Determine whether erlotinib, an EGFR/HER-1 inhibitor, could inhibit bladder cancer cell lines in vitro; (2) To identify if IFN- α could regulate EGFR expression; and (3) To estimate if combined treatment with IFN- α and erlotinib could have complementary effects. Three different experimental schedules were performed: Single drug exposure over a range of concentrations of erlotinib, combination with IFN- α , and pre-treatment with IFN- α followed by erlotinib combined with IFN- α .

[0141] Cells were grown in 24-well plates and the anti-proliferative effect was measured by crystal violet nuclear staining assay. The anti-proliferative effect of erlotinib on bladder cancer cell lines was dose-(0.10-10.00 μ g/ml) and time (0-120 h) dependent. Four cell lines, BL-17/0/X1, BL-17/2, MGHU3, and HT1376, achieved <60% of growth rate of control cells when exposed to 2.5 μ g/ml of erlotinib at 96 h point and 8 of 9 cell lines achieved <60% of growth rate of control when exposed to 10 μ g/ml. The growth inhibition correlated with the relative expression of EGFR on the cell surface ($r=0.73$). A combination of lower dose of erlotinib and IFN- α led to an enhanced anti-proliferative effect. The cooperativity quotient of the combined treatment ranged from 0.62-1.16. If the cells were first pretreated with IFN- α to initially up-regulate EGFR, prior to combined administration of IFN- α and erlotinib, then 6 of 9 of the cell lines resulted in a significantly lower growth than in the same non IFN- α pretreated cell line. The gain was from 5.82-30.37% of inhibition.

[0142] These results demonstrate that treatment with erlotinib, a potent EGFR/HER 1 inhibitor, may play a role in the treatment of bladder cancer and that combination with

IFN- α enhances the antiproliferative effect, which is further increased by IFN- α pretreatment.

[0143] Incorporation by Reference

[0144] All patents, published patent applications and other references disclosed herein are hereby expressly incorporated herein by reference.

[0145] Equivalents

[0146] Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, many equivalents to specific embodiments of the invention described specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.

What is claimed is:

1. A pharmaceutical composition comprising an EGFR kinase inhibitor and IFN α in a pharmaceutically acceptable carrier.

2. The pharmaceutical composition of claim 1, wherein the EGFR kinase comprises erlotinib, or a salt thereof.

3. The pharmaceutical composition of claim 1, additionally comprising one or more other anti-cancer agents.

4. A composition in accordance with claim 3, wherein said other anti-cancer agent is a member selected from the group consisting of alkylating drugs, antimetabolites, microtubule inhibitors, podophyllotoxins, antibiotics, nitrosoureas, hormone therapies, kinase inhibitors, activators of tumor cell apoptosis, and antiangiogenic agents.

5. A method for treating tumors or tumor metastases in a patient, comprising administering to said patient simultaneously or sequentially a therapeutically effective amount of a combination of an EGFR kinase inhibitor and IFN α .

6. The method of claim 5, wherein the patient is a human that is being treated for cancer.

7. The method of claim 5, wherein the administering to the patient is simultaneous.

8. The method of claim 5, wherein the administering to the patient is sequential.

9. The method of claim 8, wherein IFN α is administered prior to the EGFR kinase inhibitor.

10. The method of claim 8, wherein IFN α is pre-administered prior to administration of a combination of EGFR kinase inhibitor and IFN α .

11. The method of claim 5, wherein the EGFR kinase inhibitor and IFN α are co-administered to the patient in the same formulation.

12. The method of claim 5, wherein the EGFR kinase inhibitor and IFN α are co-administered to the patient in different formulations.

13. The method of claim 5, wherein the EGFR kinase inhibitor and IFN α are co-administered to the patient by the same route.

14. The method of claim 5, wherein the EGFR kinase inhibitor and IFN α are co-administered to the patient by different routes.

15. The method of claim 5, wherein the EGFR kinase inhibitor comprises erlotinib, or a salt thereof.

16. The method of claim 5, additionally comprising treating with one or more other anti-cancer agents.

17. The method of claim 16, wherein the other anti-cancer agents are selected from an alkylating agent, cyclophosphamide, chlorambucil, cisplatin, carboplatin, oxaliplatin, busulfan, melphalan, carmustine, streptozotocin, triethylenemelamine, mitomycin C, an anti-metabolite, methotrexate, etoposide, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil, capecitabine, gemcitabine, dacarbazine, an antibiotic, actinomycin D, doxorubicin, daunorubicin, bleomycin, mithramycin, an alkaloid, vinblastine, paclitaxel, docetaxel, vinorelbine, a glucocorticoid, dexamethasone, a corticosteroid, prednisone, a nucleoside enzyme inhibitors, hydroxyurea, an amino acid depleting enzyme, asparaginase, topotecan, irinotecan, leucovorin, and a folic acid derivative.

18. The method of claim 5, wherein the tumors or tumor metastases to be treated are selected from lung cancer, colorectal cancer, NSCLC, bronchioloalveolar cell lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous melanoma, intraocular melanoma, uterine cancer, ovarian cancer, rectal cancer, anal region cancer, stomach cancer, gastric cancer, colon cancer, breast cancer, uterine cancer, fallopian tube carcinoma, endometrial carcinoma, cervical carcinoma, vaginal carcinoma, vulval carcinoma, Hodgkin's Disease, esophagus cancer, small intestine cancer, endocrine system cancer, thyroid gland cancer, parathyroid gland cancer, adrenal gland cancer, soft tissue sarcoma, urethral cancer, penis cancer, prostate cancer, bladder cancer, kidney cancer, ureter cancer, renal cell carcinoma, renal pelvis carcinoma, mesothelioma, hepatocellular cancer, biliary cancer, chronic leukemia, acute leukemia, lymphocytic lymphoma, CNS neoplasm, spinal axis cancer, glioma, brain stem glioma, glioblastoma multiforme, astrocytoma, schwannoma, ependymoma, medulloblastoma, meningioma, squamous cell carcinoma and pituitary adenoma tumors or tumor metastases, including refractory versions thereof.

19. The method of claim 18, wherein the tumors or tumor metastases to be treated are colorectal cancer tumors or tumor metastases.

20. The method of claim 18, wherein the tumors or tumor metastases to be treated are bladder cancer tumors or tumor metastases.

21. A method for the treatment of cancer, comprising administering to a subject in need of such treatment a first amount of an EGFR kinase inhibitor, or a pharmaceutically acceptable salt thereof, and a second amount of IFN α wherein at least one of the amounts is administered as a sub-therapeutic amount.

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